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STUDIES OF THE CORNEAL NERVE CANAL AS A WAY OF LIQUID CURRENT AND THE OCCURRENCE OF PUNCTATE OPACITIES IN THE CORNEA

Kaichi WAKUI

Department of Ophthalmology, Branch hospital of the Univ. of Tokyo

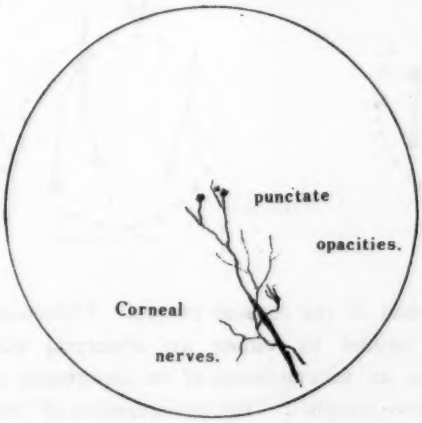
There is a problem about the occurence of punctate opacities on the cornea. The punctate opacities were seen mainly with epidemic keratoconjunctivitis, allergic conjunktivitis, keratitis nummularis, and a type of herpetic keratitis. In 1930, R. E. Wright[20] had the punctate opacities in the rabbit's eye, and in 1942, K. Suda[21] caused the opacities in rabbit's eye by inoculation of Staphylococcus aureus. But, the mechanism of the occurrence was not explained, with which the author has tried the following experiments, getting the punctate corneal opacities in the rabbit's eye.

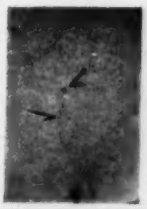
I. The Corneal Nerve Canal as a Way of Liquid Current, and the Occurrence of Punctate Opacities in the Cornea

The punctate corneal opacities of the rabbit's eye (see chapter III) were seen at the tip or at the surrounding fine branches of the corneal nerves. Was it accidental or not? If they always appear at the tip of the branches, there might be some relations in their mechanism (between the opacities and the nerve branches). The observations were made along this line.

Then, several cases of rabbit's eye, 40 cases of epidemic keratoconjunctivitis, and several cases of the other diseases were observed. Both in the rabbit's eye (see Fig. 1) and in the human eye (see Fig. 2,3.), the opacities were usually seen at the tip

Fig. 1. Punctate corneal opacities of the rabbit's eye (this photograph is negative). No. 2.





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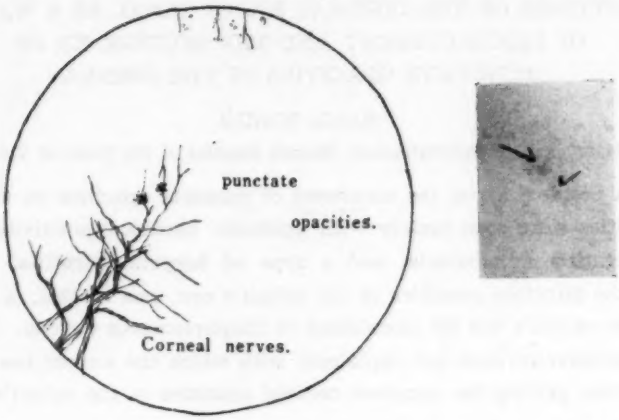

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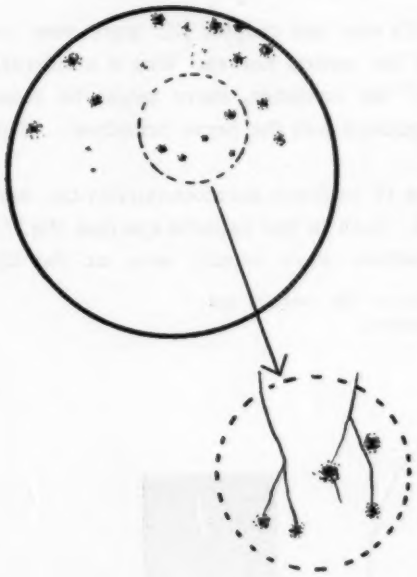

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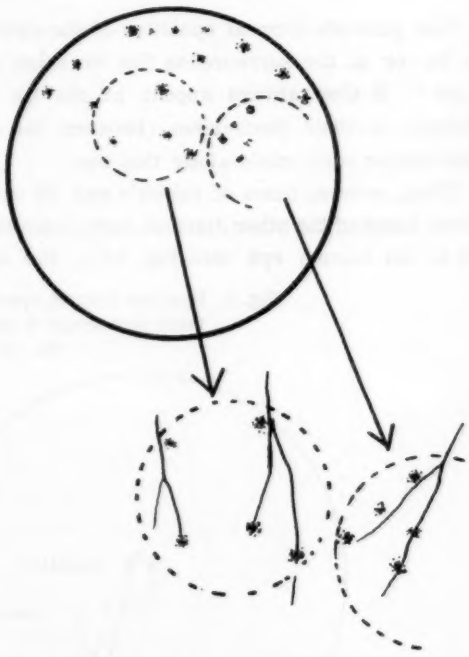


or at the surrounding fine branches of the corneal nerves. Therefore the author thought that the opacities were caused by either an abnormal stimulus acting through the corneal nerve, or by an accumulation of an abnormally produced fluid brought on through the corneal nerve canals[1]. The transmission of the stimulus is

functioned by the nerves, but the existence of the nerve canal as a way of liquid current is an unknown fact[1)2)3)4)5)6)7)8)9)10)11)12)].

Method and Results

Full grown white rabbits were used.

1) *Methylene blue was injected in the subconjunctival near the limbus.*

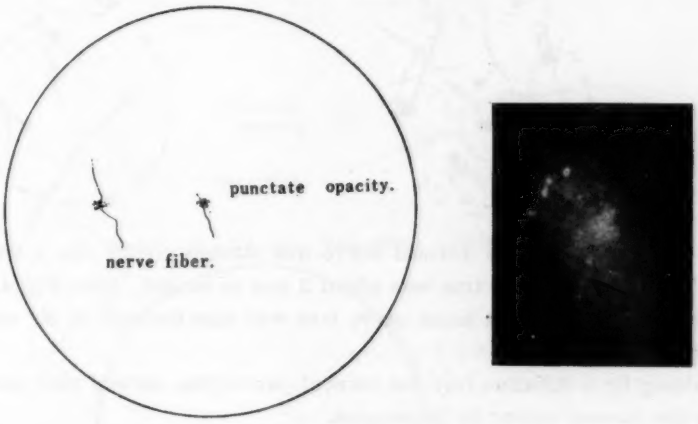
30 minutes later, one of the little nerve branches was stained at the limbus. (See Fig. 4.1)

With Fluorescin, nerves were not stained. (See Fig. 4.2)

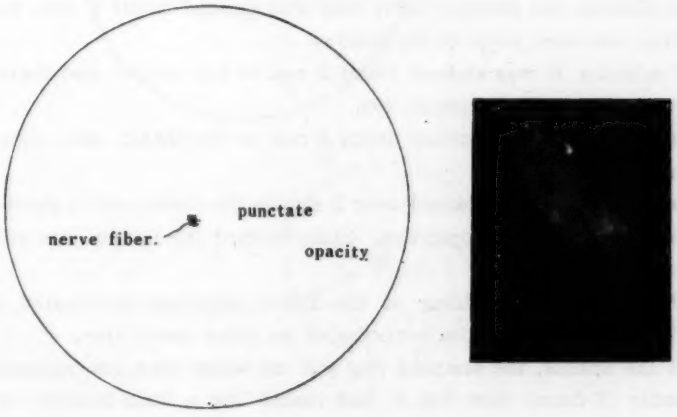
2) *Methylene blue was injected into the parenchyma of the limbus.*

Fig. 2. Punctate corneal opacities of the cases of epidemic keratoconjunctivitis (this photograph is positive).

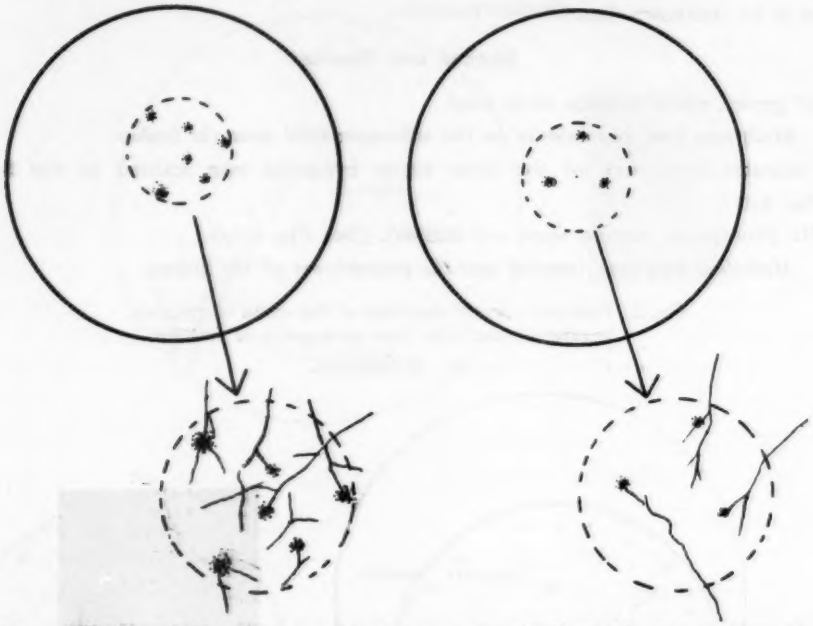
No. 651. 33 years old.



No. 3521. 45 years old.



No. 3833. 26 years old. No. 4584. 27 yearsld.



Thirty minutes later, the corneal nerve was stained vividly like a tree with fine branches. This stained nerve tree was about 3 mm in height. 〔See Fig. 4.3)〕

With Fluorescin, the 3 mm heigh nerve tree was also stained in 30 minutes, but it had no branches. 〔See Fig. 4.4)〕

The staining by a diffusion into the corneal parencyma showed only one millimeter in width to the corneal center in 30 minutes.

3) *Methylen blue was injected into the overwall of the nerve tree bough.* (*See Fig. 5.*)

After 10 minutes, the corneal nerve tree was stained about 1 mm to the center, but no staining was seen towards the limbus.

After 20 minutes, it was stained about 2 mm to the center, and there was a slight staining towards the limbus vestigial too.

After 30 minutes, it was stained about 3 mm to the center, and about 1mm towards the limbus.

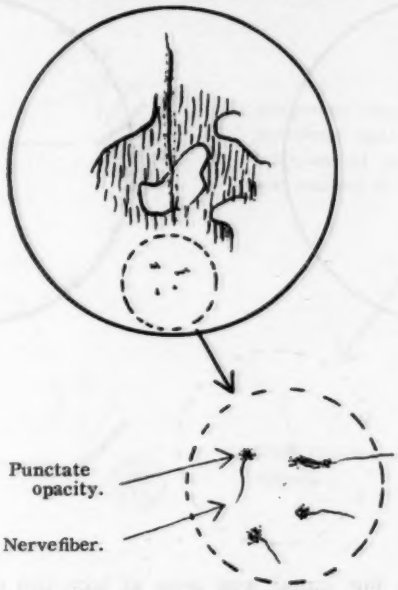
After 40 minutes, it was stained over 3 mm to the center, and a tip of the branches reached one of the punctate opacities. Stain toward the limbus was still only 1 mm but the colour was deeper.

After 60 minutes, the staining of the nerve branches penetrated the punctate opacity and started to invade the branches of an other nerve tree.

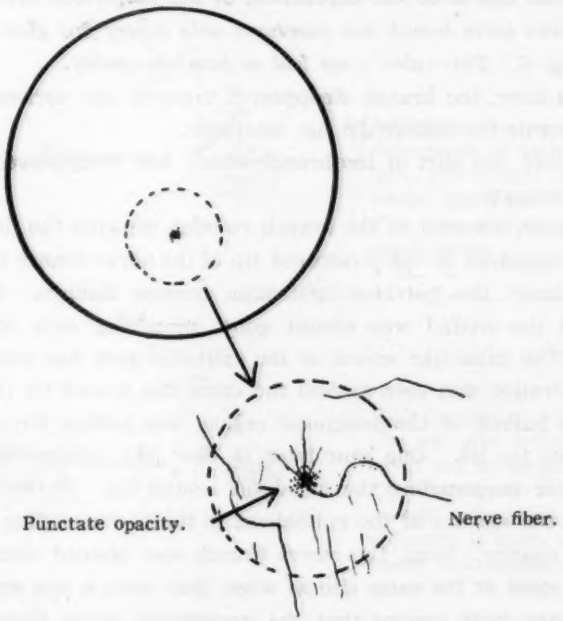
Towards the limbus, the staining was still no wider than one milimeter.

This opacity (E-form) (See Fig. 5) had looked like a little bladder by the recur-

Fig. 3. Punctate corneal opacities those were seen in the cases of several diseases of the human eye.
No. 4355. 61 years old. Herpetic keratitis.

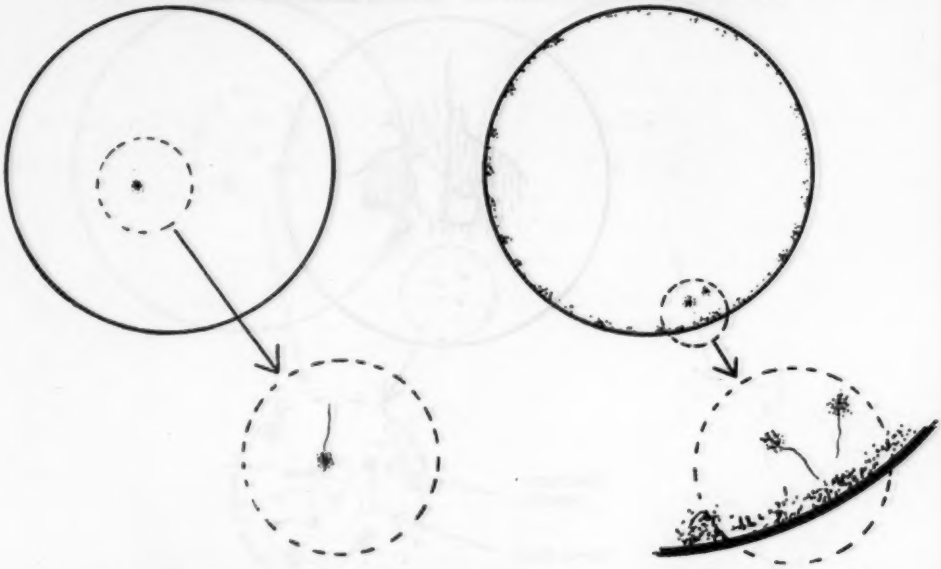


No. 442. 25 years old. Iritis serosa. (Diabetes mellitus)



N4. 3913. 49 years old. Keratitis epithelialis punctata. (Sugiura)[27]

No. 1697. 13 years old. Allergic Conjunctvitis.



rence ray. A very fine canal was seen at both tips of the bladder. This bladder grew larger at both tips after the experiment of the methylene blue injection.

4) *A nonstained nerve branch was punctured with a very fine glass needle of 40μ in diameter. (See Fig. 6.) This rabbit's eye had no punctate opacity.*

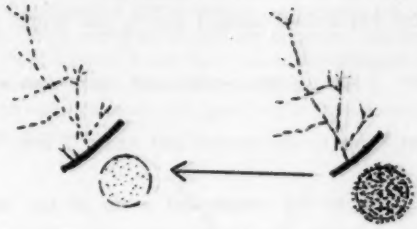
Two minutes later, the branch disappeared towards the corneal center, but the part running towards the limbus did not disappear.

15 minutes later, the part of the branch which had disappeared appeared again, although it was delicate.

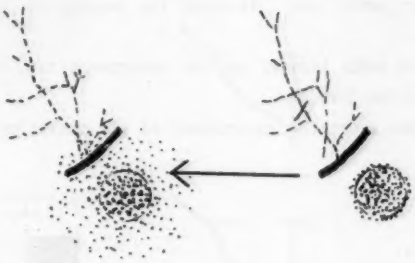
30 minutes later, the part of the branch running towards the limbus became thin. Mild infiltration appeared at the punctured tip of the nerve branch towards the limbus.

60 minutes later, the previous infiltration become thinner. And 3 days later, the opacity from the wound was almost gone, remaining only at the surrounding nerve branch. The canal-like wound of the epithelial part was completely recovered, though mild infiltration was seen around the canal-like wound tip in the subepithelial layer. Then the bulbus, at the punctured region, was pushed strongly by a finger tip several times over the lid. One hour later, a disc like infiltration appeared at the subepithelial layer surrounding the canal-like wound tip. Moreover, a mild infiltration occurred at the surface of the epithelium to the corresponding site of the center of that disc-like opacity. Next, this nerve branch was stained with methylene blue, the nerve fibers stood at the same site as when they were a non stained visible nerve branch. (Thus one could assume that the nonstained nerve fibers are not visible.

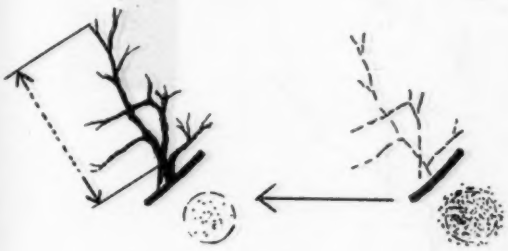
Fig. 4.



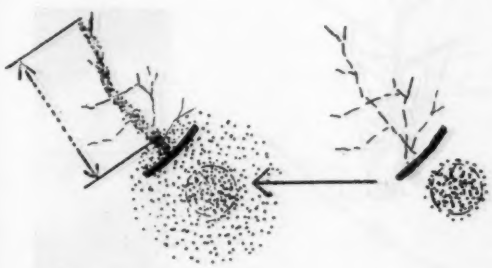
1) Methylene blue was injected subconjunctivaly near the limbus. 30 minutes later, one of the little nerve branches was stained at the limbus.



2) With Fluorescin, nerves were not stained.



3) Methylene blue was injected into the parenchyma of the limbus. 30 minutes later, the corneal nerve was stained vividly like a tree with fine branches. This stained nerve tree was about 3 mm in height.



4) With fluorescin, the 3 mm heigh nerve tree was also stained in 30 minutes, but it had no branches.
The staining by a diffusion into the corneal parenchyma showed only one milimeter in width to the corneal center in 30 minutes.

Fig. 5. Methylen blue was injected into the overwall of the nerve tree bough.

1) After 10 minutes, the corneal nerve tree was stained about 1 mm to the center, but no staining was seen towards the limbus.

2) After 20 minutes, it was stained about 2 mm to the center, and there was a slight staining towards the limbus vestigial too.

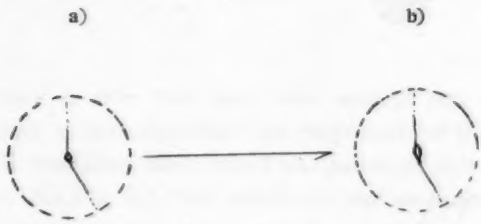
3) After 30 minutes, it was stained about 3 mm to the center, and about 1 mm towards the limbus.

4) After 40 minutes, it was stained over 3 mm to the center, and a tip of the branches reached one of the punctate opacities. Stain towards the limbus was still only 1 mm but the colour was deeper.

After 60 minutes, the staining of the nerve branches penetrated the punctate opacity and started to invade the branches of an other nerve tree. Towards the limbus, the staining was still no wider than 1 mm.

a) This opacity (E-form) had looked like a little bladder by the recurrence ray. And a very fine canal was seen at the both tips of the bladder.

b) This bladder grew larger at both tips after the experiment of the methylen blue injection.



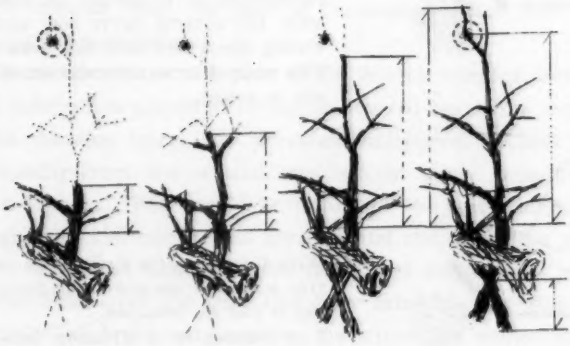
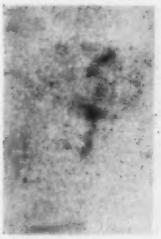


Fig. 6.

1) A nonstained nerve branch was punctured with a very fine glass needle of 40 μ in diameter. (This rabbit's eye had no punctate opacity.)

2) Two minutes later, the branch disappeared towards the corneal center, but the part running towards the limbus did not disappear.

3) 15 minutes later, the part of the branch which had disappeared appeared again, although it was delicate.



2)

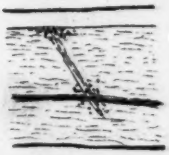
(This photograph is negative.)

1)

2)

3)

4)



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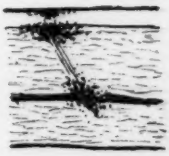

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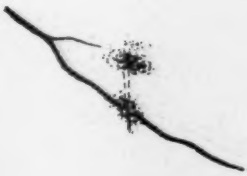


6)

1)

4) 30 minutes later, the part of the branch running towards the limbus became thin.

Mild infiltration appeared at the punctured tip of the nerve branch towards the limbus.

5) 3 days later, the opacity from the wound was almost gone, remaining only at the surrounding nerve branch. The canal like wound of the epithelial part was completely recovered, though mild infiltration was seen around the canal-like wound tip in the subepithelial layer.

6) Then the bulbus, at the punctured region, was pushed strongly by a finger tip several times over the lid. One hour later, a disc like infiltration appeared at the subepithelial layer surrounding the canal-like wound tip. Moreover, a mild infiltration occurred at the surface of epithelium to the corresponding site of the center of that disc-like opacity.

Fig. 7.

0.5% hematoxylin normal saline solution was injected into the parenchyma at the limbus. Nerve canals were stained vividly like a tree 6 to 8 mm, in height. Then, the staining stopped and began to disappear.

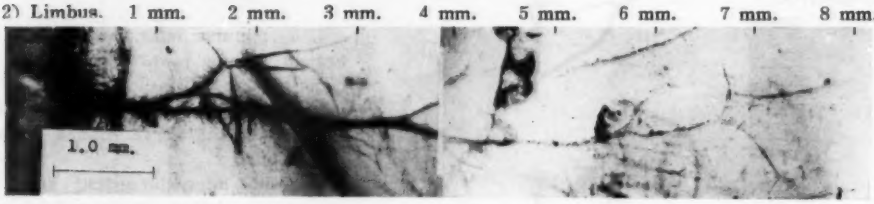
1) (Normal eye) The solution reached at 6 mm distant from the limbus. The canal wall was stained, and the hematoxylin was accumulated at the little branches. No wandering cells were seen in the canal's inner space.

2) (Inflammatory eye) The solution reached at 8 mm distant from the limbus. The canal was stained, and no accumulation was found.

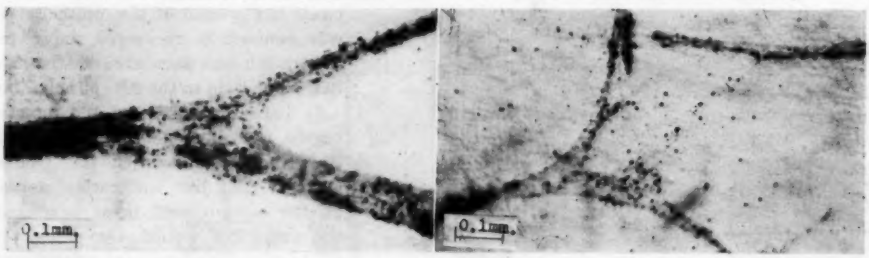
3) (Inflammatory eye) In the canal's inner space many wandering cells were seen, and some of them were floating surrounding the little branches.

1) (3 to 4 mm. from the limbus) (4 to 5 mm.) (5 to 6 mm.)

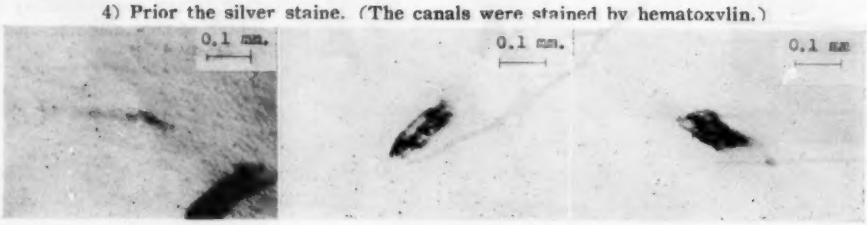
2) Limbus. 1 mm. 2 mm. 3 mm. 4 mm. 5 mm. 6 mm. 7 mm. 8 mm.



3) (3 to 4 mm. from the limbus) (7 to 8 mm. from the limbus)



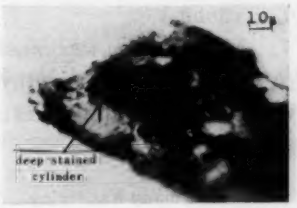
4) Prior the silver staine. (The canals were stained by hematoxylin.)



5) After the silver staine.

6) After the silver staine.

The canals of normal rabbit's eye, which were stained by hematoxylin vital staining, were stained according to the Suzuki's silver stain method. The canal wall was stained relatively deep. And at the center of the canal a deep stained cylinder was found.



So the nerve branch which is visible without staining is not a nerve fiber, it is a nerve canal. The nerve canals are visible, when the canal current exists, and becomes invisibie when the current is stopped.)

5) *0.5% hematoxylin normal saline solution was injected into the parenchyma at the limbus.* (*See Fig. 7.*)

Nerve canals were stained vividly like a tree 6 to 8 mm in height. Then the staining stopped and began to disappear. Fig. 7 is one of them. The nerve canal wall was stained by hematoxylin, and the parenchyma was invaded with hematoxylin along the nerve canals. The nerve canal bough ran in the deep layer from the limbus to the corneal center, and towards the corneal surface.

a) Hematoxylin solution was injected into the parenchyma at the limbus of the normal rabbit's eye. The solution reached 6 mm distant from the limbus. The canal wall was stained, and the hematoxylin was accumulated at the little branches. No wandering cells were seen in the canal's inner space. [See Fig. 7.1)]

b) Hematoxylin solution was injected into the parenchyma at the limbus of the inflammatory eye (caused by alkali solution dropped in the eye).

The solution reached 8 mm distant from the limbus. The canal was stained, and

no accumulation was found. In the canal's inner space, many wandering cells were seen, and some of them were floating surrounding the little branches. 〔See Fig. 7.2)3)〕

c) The canals of normal rabbit's eye, which were stained by hematoxylin vital staining, were stained according to the Suzuki's silver stain method.

The canal wall was stained relatively deep. At the center of the canal a deep stained cylinder was found. 〔See Fig. 4)5)6)〕

d) The nerve canals in the parenchyma of the sclera.

The nerve canals were also stained in the parenchyma of the sclera by hematoxylin vital staining. This canal was found at the deep layer of the sclera, 〔Fig. 8.1) c.〕 running in the neighbourhood of the blood vessels.

〔Fig. 8.1) b.: The deep stained shadow is the nerve canal wall, and the labyrinthlike canals are the blood vessels.〕

6) *We refer to the histological observations of the rabbit's eye in which the punctate opacities were caused.* There are many wandering cells in the deep layer of the sclera in the neighbourhood of the limbus. 〔Fig. 8.1) a.〕

They are the results of an antigen-antibody reaction in subconjunctival space. (No. 21 rabbit.)

7) *Methylen blue was injected prior to the enucleation at the limbus of the human eye which had glioma.* The methylen blue solution reached the subepithelial fine corneal nerve canals, when at times the solution invaded the very fine epithelial canals 〔Fig.

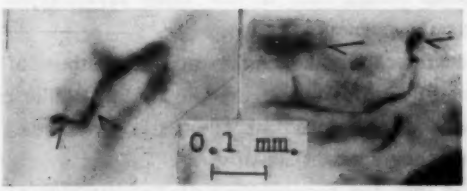
Fig. 8

1) a. There are many wandering cells in the deep layer of the sclera of No. 21 rabbit.

b. The deep stained shadow is the nerve canal wall, and labyrinthlike canals are the blood vessels.

c. The nerve canal was found at the deep layer of the sclera. (3 mm from the limbus.)

2) a. b.



2) Methylen blue was injected prior the enucleation at the limbus of the human eye which had glioma.

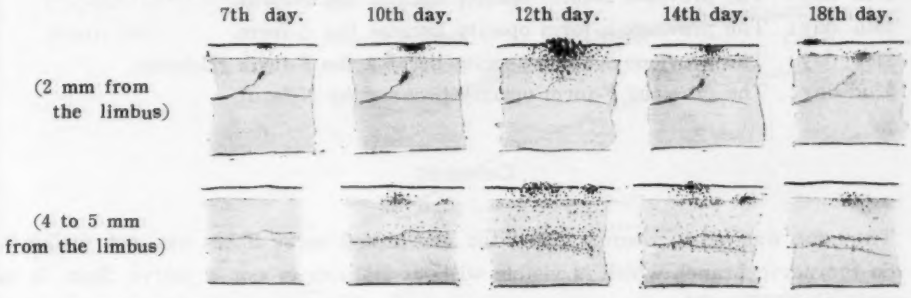
The methylen blue reached at the subepithelial fine corneal nerve canals.

a. At times the solution invaded the very fine epithelial canals.

b. At times the solution was accumulated there.

3) The punctate opacities appearing in the cases fo epidemic keratoconjunctivitis.

No. 548. 38 years old woman.



No. 2804. 55 years old man.

8.2) a.〕, and at times it was accumulated there 〔Fig. 8.2) b.〕.

8) *The punctate opacities appearing in the cases of epidemic keratoconjunctivitis, were observed.* 〔Fig. 8.3)〕

(With the classification of types of the punctate opacities, refer to the paragraph of chapter III).

No. 548. 38 years old woman.

7th day: B-form opacity appeared at 2 mm from the limbus, but a fine nerve canal branch was found under the opacity. At the tip of this fine branch slight infiltration was found.

10th day: C-and F-form opacities appeared at 4 to 5 mm from the limbus. The nerve canals running under the opacities had slight infiltration.

12th day: At 2 mm distance from the limbus, previous B-form opacity became the A-form. At 4 to 5 mm distance from the limbus, previous C-, and F-form opacity became the A-form.

14th day: The opacities became milder, but the infiltration of the nerve canals became more significant.

18th day: At 2 mm distance from the limbus, previous A-form opacity became the F-form (U-form), and slight infiltration remained at the tip of the nerve branch. At 4 to 5 mm distance from the limbus, the previous A-form opacity became the F-form (U-form). The infiltration of the nerve canals also became mild.

No. 2804. 55 years old man.

This case was observed in 1957, when the author had no knowledge of the corneal nerve canals.

15th day: D-form opacity appeared at 4 to 5 mm distance from the limbus.
17th day: The previous D-form opacity became the E-form.
18th day: The previous E-form opacity became the A-form.
21st day: The previous A-form opacity became the F-form (U-form).
32nd day: The previous F-form opacity became the N-form.

Comment

Thus one was led to assume that "the nonstained nerve fibers are not visible".

So the nerve branch which is visible without staining is not a nerve fiber, it is a nerve canal[9)13)]. The nerve canals are visible, when the canal current exists, and becomes invisible, when the current is stopped. And these nerve canals are seen in both, the rabbit's eye, and in the human eye.

In some human cases the nerve canals were seen not only at the large bough but also at the very fine epithelial branches. Then the existence of a circulation system in the corneal parenchyma was realized; which the author wishes to call the nerve canal system. Through this nerve canal, the liquid current runs at the speed of about 1 mm in 10 minutes towards the corneal center at the principal bough, but slower at the smaller branches.

The current becomes more rapid when any stimulus attacks the cornea.

The punctate opacities of the rabbit's eye seems to be an infiltration caused by the accumulation of the liquid, that was carried through the corneal nerve canals. (See Fig. 1.5.6.7.8.9.). But it does not mean that the other factors, (for example, unidirectional liquid movement in the corneal parenchyma—; see chapter II), should have no relation to the occurrence of punctate opacities.

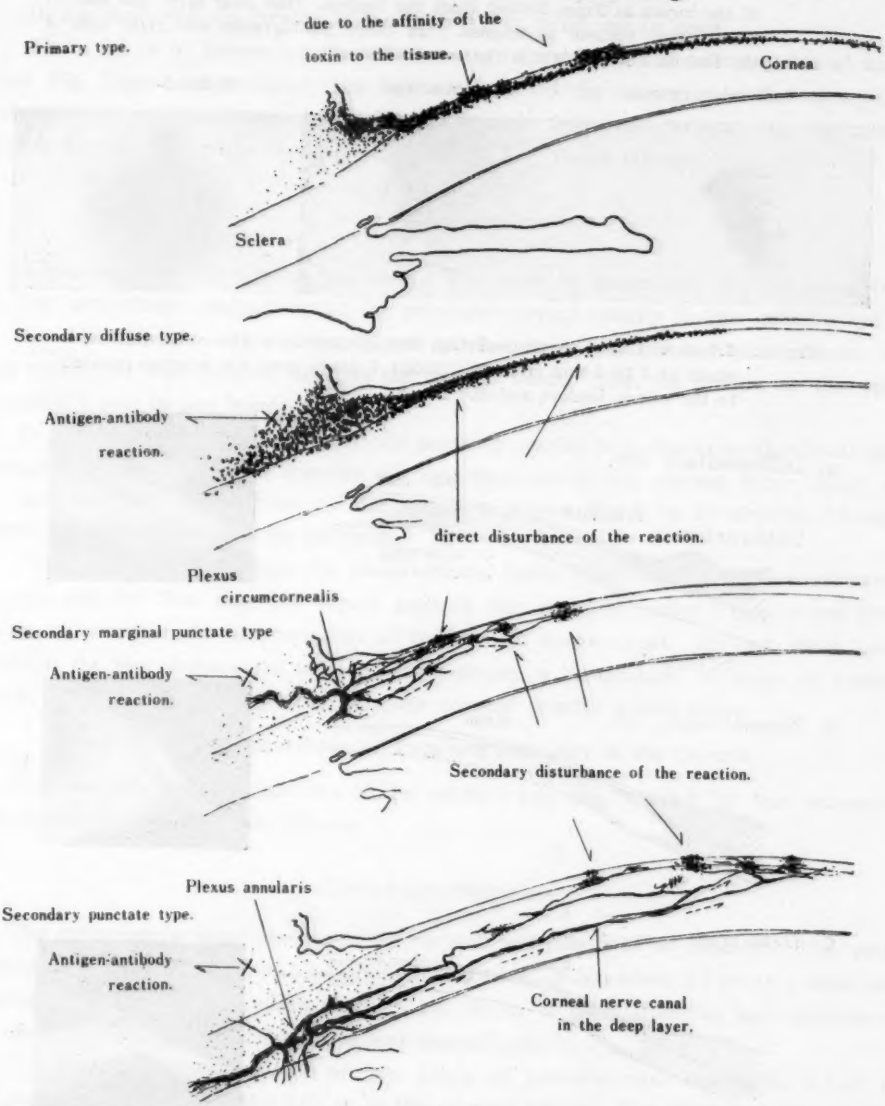
According to anatomy[2)], the corneal nerves in the superficial layer at the limbus run from the plexus circumcornealis, mainly to the marginal area.

The nerves in the deep layer at the limbus run from the plexus annularis, mainly to the central regions. The plexus circumcornealis is located at the subconjunctival to episcleral tissue, and the plexus annularis is located in a relatively deep layer of the sclera. It seems therefore, that the disturbances at the deep layer in the pericorneal region is the cause for the punctate opacities in the central region of the cornea, and in the same way the disturbance at the superficial layer in the pericorneal regions is the cause for the opacities in the marginal area. (See Fig. 9.)

II. The Liquid Movement in the Corneal Parenchyma and the Punctate Opacities in the Cornea

The liquid movement in the corneal parenchyma is important for considering the occurrence mechanism of the punctate corneal opacities.

Fig. 9. Occurrence mechanism of the corneal changes.



Many authors had reported on the liquid movement and the liquid pass way in the cornea[5)6)11)12)14)15)16)17)18)19]. With the same purpose, the author reported the corneal nerve canal system, and has tried the following experiments.

Method and Result

Full grown white rabbits were used.

Fig. 10. 0.5% hematoxylin normal saline solution was injected into the parenchyma of the cornea at 3 mm distant from the limbus. One hour later, the spot of the solution became as follows. (In these photographs, the right side is the limbus, and the left is the corneal center).

a. Inflammatory eye. b. Normal eye. c. Dead eye.

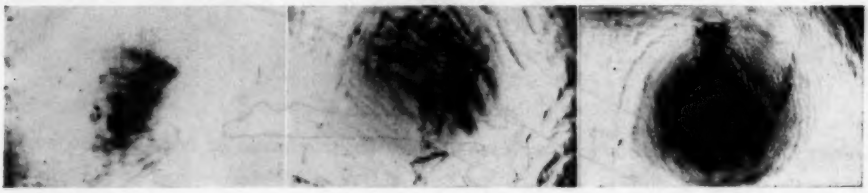
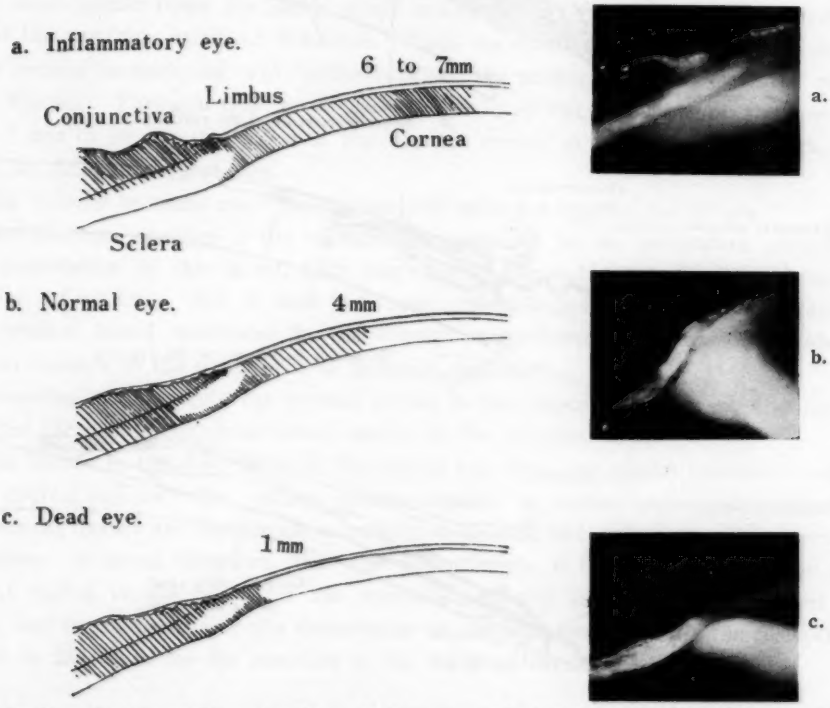


Fig. 11. 0.5 cc of lithion-carmin solution was injected into the subconjunctival space at 3 to 4 mm from the limbus. 4 hours later, the solution invaded to the sclera, limbus, and the cornea.



A little quantity of hematoxylin solution was injected into the parenchyma of the cornea at 3 mm from the limbus. (See Fig. 10.)

In the cases of the inflammatory eye (in living rabbits), it became visible after 30 minutes, that the center of the spot of the injected solution moved towards the corneal center. In the normal eye (in living rabbits), it became visible after one hour, but

it was merely vestigial.

In the dead eye (in freshly killed rabbits), it could not be seen.

Then, 0,5 cc of lithion-carmin solution was injected into the subconjunctival space. (See Fig. 11). 4 hours later, the solution invaded the cornea. In the cases of the inflammatory eye, it measured 6 to 7 mm distance from the limbus. In the normal eye, it was 4 mm, and in the dead eye, it was only 1 mm distant.

Comment

Through these experiment, the author was able to appreciate the following facts on the occurrence mechanism of the punctate corneal opacity in the rabbit's eye.

1) There is an unidirectional liquid movement in the corneal parenchyma, and this movement was directed towards the corneal center from the limbus at the speed of under 1 mm in one hour.

2) There are two way to reach the punctate opacity from the subconjunctival space through the sclera. One is through the liquid current of the corneal nerve canal, and by this way the liquid runs at the speed of about 1 mm in 10 minutes, having a higher concentration than the following.

The other way is through the unidirectional liquid movement in the corneal parenchyma, and by this way the liquid goes at the speed of under 1 mm in one hour, acting surrounding the fine branches of the corneal nerve canal. If any weak point exists at the fine nerve canal branch; for example, a bifurcation, a point of anastomosis, or a subepithelial tip; the punctate corneal opacity would occur.

III. The Occurrence of Punctate Opacities in the Cornea

The punctate corneal opacities of the rabbit's eye were caused by the successive subconjunctival injection, as follows.

Method and Materials

The experiments were performed by various methods with 66 rabbits. The main method was the repeated subconjunctival injection of inoculum 0.2 cc at a time performed every other day, seven times in all. Six or 10 days after the last injection of the main method, the second method was carried out.

The second method consisted of two kinds of provocational injections, 0.2 cc in the subconjunctiva, and the 0.02 cc in the corneal tissue. The third method was the control of the second method, which was the same as the second method, but had no repeating of subconjunctival injection. The fourth and the fifth method followed. The fourth method was " the 3-week-observation " of the eye which received one injection into the corneal tissue, and the fifth method was a subconjunctival scarification of episcleral tissue. Of course, they had no repeating of subconjunctval injection.

Inoculum

1) On the second to the fourth method the inoculums consisted of two kinds; one was the inoculum of Adenovirus type 8, and the other was the inoculum of He-La cell tissue culture without virus. The pH. of the He-La cell tissue culture without virus was measured many times during its cultivation, so as to match it to the pH.

Fig. 12. Corneal changes; those were caused by the successive subconjunctival injection. —Shapes by slitlampmicroscopy—

1. Primary type. (Diffuse subepithelial infiltration; those accompanied at times the epithelial mass infiltration.)
2. Secondary diffuse type. (Diffuse subepithelial infiltration; those did not accompany the epithelial mass infiltration.)
3. Secondary marginal punctate type. (Punctate infiltrations on the margin of the cornea.)
4. Secondary punctate type. (Punctate infiltrations those had a distance more than 2 mm from the limbus.)

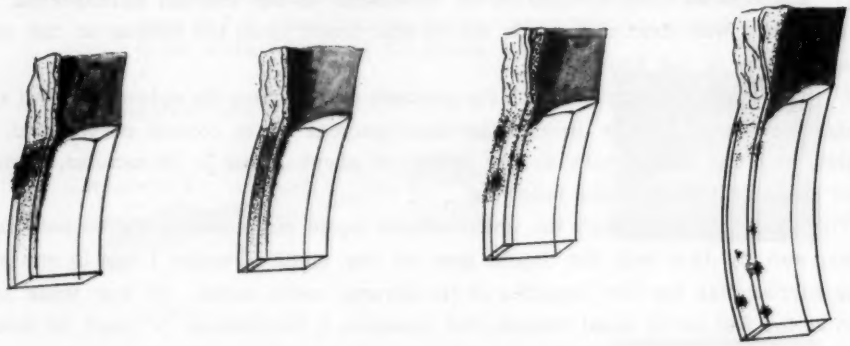
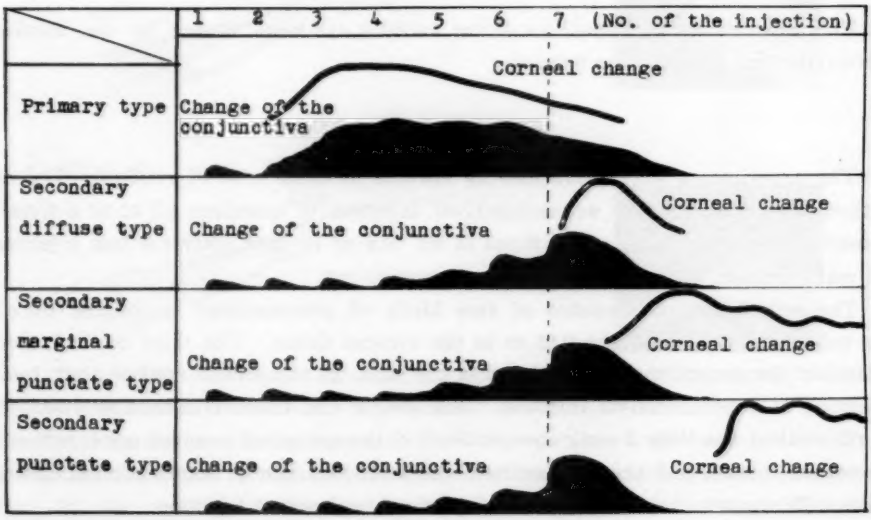


Fig. 13. Corneal changes; those were caused by the successive subconjunctival injection. —Course of the corneal changes—

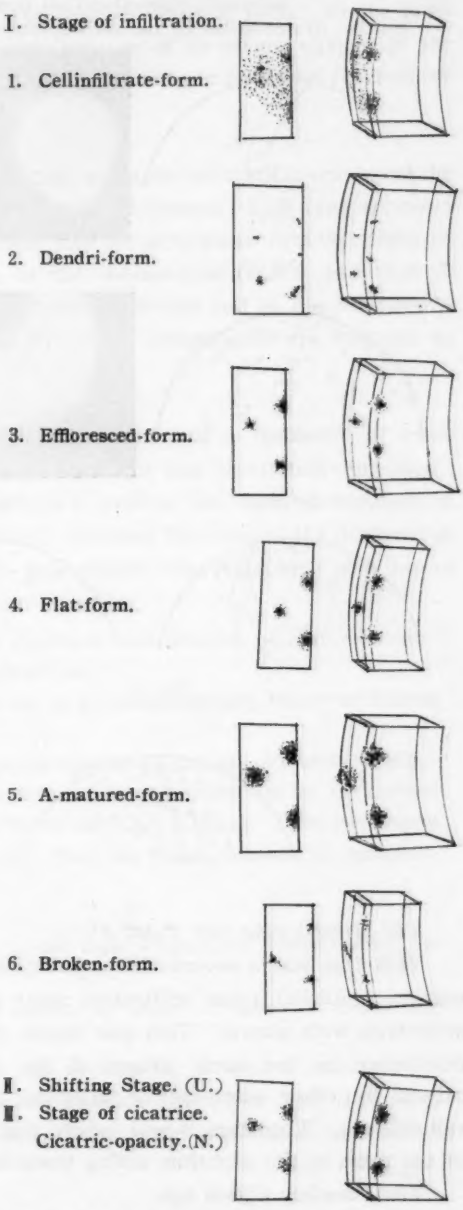


of the inoculum of Adenovirus type 8. The Adenovirus type 8 inoculum measured 7.00 of pH , and the He-La cell injection, 7.01 (They were measured after keeping it at 56°C for 30 minutes). The total nitrogen in the inoculum of the Adenovirus type 8 was detected 96.8 mg/dl, and in the He-La cell injection, showed 69.3 mg/dl. The Strain (Trim) was sent by Huebner Laboratory to the Institute for Infectious Diseases (Univ. of Tokyo), and from there brought to our laboratory. After inoculating 0.15 cc of this inoculum, He-La cell tissue culture showed three plus cytopathogenic changes in three days.

2) On the main method, in addition to these inoculum, Adenovirus type 3, purified vaccine virus and bovine serum were used.

The strain of Adenovirus type 3 was the prototype sent from the Huebner Laboratory. After inoculating 0.1 cc of this inoculum, He-La cell tissue culture showed three plus cytopathogenic changes in two days. This inoculum measured 6.60 with pH., while the control injection of He-La cell tissue culture was 7.05. As the purified vaccine virus, Yaoi antigen was used with no dilution. Bovine serum was used also with no dilution. By inoculating this bovine serum, He-La cell tissue showed no cytopathogenic changes. It means that almost no virus exsisted in this bovine serum.

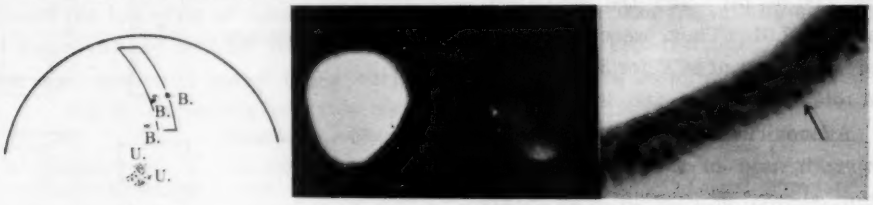
Fig. 14. Classification of the punctate opacities.



Results

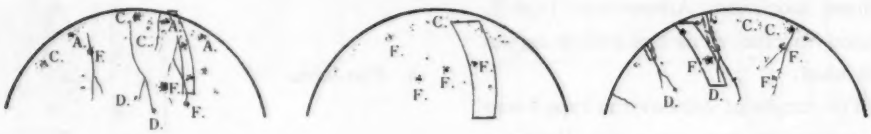
The author obtained and classified four types of corneal changes that were seen during the successive subconjunctival injection (the main method). (See Fig. 12, 13).

Fig. 15. Punctate corneal opacities of the rabbit's eye, those were caused by the successive subconjunctival injection of Adenovirus type 8.
No. 15. (Observations by the slitlampmicroscopy) (Histological observations)



No. 16.

No. 21. No. 23. No. 24.



No. 29. No. 32.

The primary type (*See Table 3*).

This type was a severe diffuse subepithelial infiltration, at times accompanied with marked epithelial mass infiltration many times with penetrating of blood vessels, and sometimes with ulcers. This was found only with the purified vaccine virus group, developing in the early stages of the repeated inoculation. This type was not caused by other inoculum or injection, that had no affinity of the toxin to the rabbit's eye. Therefore it was judged that this type was caused mainly by the affinity of the toxin in the inoculum acting towards the tissue of the rabbit's eye.

The secondary diffuse type

This type was marked diffuse subepithelial infiltration, that had neither the focus of epithelial infiltration nor the ulcer. This type developed from the limbus 12 to 24

hours after the last inoculation (injection), and was seen mainly in the bovine serum group. In the Adenovirus group this type was seen in vestigial form. In the purified vaccine virus groups, this type was not seen because of its severe change of the primary type. It was judged that this type was due to antigen-antibody reaction of subconjunctival injection.

The secondary marginal punctate type

This type was subepithelial, at times epithelial, punctate infiltration, occupying the marginal site of the cornea within 2 mm distance from the limbus. This type appeared more than 48 hours after the last inoculation, and its occurrence rate was 100% in the bovine serum group and 70 to 90% with in the Adenovirus type 8 and type 3. There was no difference between the cases of bovine serum and of the Adenovirus group. It was also judged, that this type was due to an antigen-antibody reaction of the subconjunctival injection.

The secondary punctate type (*See Table 4*).

This type was a subepithelial punctate infiltration that had a distance* of more than 2 mm from the limbus, appearing 5 to 6 days after the last inoculation (injection). This type appeared in 41% of the Adenovirus type 8, and in the same percentage of the bovine serum group. There was no difference between the case of the Adenovirus type 8 and of bovine serum. Therefore it was judged that this type was due to antigen-antibody reaction of the subconjunctival injection.

* Only the opacities, that satisfy these seven conditions were judged as punctate opacities.

1) Those that cannot be found prior the inoculations.

2) Those that can be found both with loupe and by slitlampmicroscopy, before the staining with fluorescin.

3) Excluding the opacities, those are like keratitis superficialis diffusa. (Keratitis superficialis diffusa; Fine dot-like staining by fluorescin, or fine grayish white infiltrates of the corneal surface, are usually called KERATITIS SUPERFICIALIS DIFFUSA in Japan. After the reports by Sjögren, keratitis sicca was separated from them. Then, this disease consists of Ariboflavinosis and other unknown facts).

4) Those larger than 0.04 mm in diameter.

5) Excluding those appearing on the periphery; and those within 2 mm distance from the limbus.

6) Excluding those that appear only on the center, (by center is meant to specify the 2 mm in diameter).

7) Excepting the opacity that appeared alone with no following outbreak of opacities.

Classification of the punctate opacities (*secondary punctate type*). (*See Fig. 14, 15*).

The punctate opacities which satisfied the above seven conditions consisted of various types as follows.

a. Stage of infiltration.

1) Cell-infiltrate-form (Cellinfiltrations-Form).

Focal infiltration occupied either the subepithelial or superficial, and many times

both regions. Its shape could be scarcely discerned because of the existence of the accessory infiltration, but the form was rather round.

Accessory infiltration also occupied subepithelial or superficial, and at many times the both regions. These accessory infiltrations were so diffused, that they had no shapes nor ridges.

The surface was flat but rough. In many cases the surface was stained with fluorescin, but some of them almost had no changes.

It was called C-form, and was found in almost every rabbit's eye which had punctate opacities. It was mostly found in the neighbourhood of the limbus. C-form is written as a cellinfiltrate form, but not all cases had cellinfiltration. It merely means that the C-form seems like a cellinfiltration when seen by slitlampmicroscopy.

2) Dendri-form (Dendriten-Form).

Focal infiltration occupied subepithelial or the superficial tissue of the corneal parenchyma. Its shape seemed like a piece of a naked branch.

Accessory infiltration could not be found as a rule.

The surface had no changes, but in a few cases, fluorescin stain was seen.

It was called D-form, and was found in the rabbit's eye of No. 21 and 24.

It was found in the middle zone between the center and the limbus of the cornea.

3) Effloresced-form (Eckige-Form).

Focal infiltration occupied subepithelial tissue. Its shape was polygonal, like a star or a flower.

Accessory infiltration was found at many times as a slight infiltration on the superficial tissue of the epithelium.

The surface was flat, but at many times it was stained with fluorescin.

It was called E-form, and was found in the rabbit's eye of No. 16 and 32.

In many cases D- and C-form became E-form.

4) Flat-form (Flache-Form).

Focal infiltration occupied the subepithelial tissue. Its shape seemed like a little disc or a flat ball.

Accessory infiltration was the same as in E-form.

The surface was the same as in E-from.

It was called F-form, and these were found in almost every rabbit's eye under examination. At many times usually the E-form became F-form, but at times it seemed that the C-form became F-form.

5) A-matured-form (Angereifte-Form).

Focal infiltration occupied the subepithelial tissue. Its shape seemed like a flat ball. Most times the center of the flat ball swelled like a dull spindle into the corneal parenchyma, and the other tip swelled into the epithelial tissue.

Accessory infiltration was always senn in the superficial tissue of the epithelium.

The surface always swelled from the plane surface, and in almost all cases fluorescin-

stain was detected.

The above type was called A-form, and was found in rabbit's eye of No. 16, 21,29. They were mostly seen after the F-form but few times after the C-form.

6) Broken-form (Bruch-Form).

Focal infiltration always occupied the epithelial tissue, never the subepithelial. Its shape varied, but it was often smaller than the other forms.

Accessory infiltration, as a rule, could not be seen.

The surface always seemed rough, and many times it was stained with fluorescin.

It was called the B-form, and was found in many cases of the rabbit's eye at the initial stage of the punctate opacities. The B-form usually took various courses. At times it grew to C,D,E,F, and at times it disappeared directly from the B-form.

b. Shifting Stage.

The full grown type of the punctate opacities is the A-form. After that (A-form), punctate opacities began to disappear. At the first, the swelling of the surface become flat, and next the accessory infiltrations disappeared. Then, the infiltrations of the focal infiltration become thinner.

These stages were called the shifting stage. The punctate opacities of these stages were called U-form (übergangs-Form). The U-form was found in rabbit's eye of No. 15,16,21,23.

c. Stage of cicatrice.

At the shifting stages, the infiltrations were at times decreased, and at times increased. But in time, the infiltrations become fixed. This fixed stage will be called the stage of cicatrice. It was called the N-form (Narben punctata), but it could not be found in the rabbit's eye.

Comment

Thus it led to consider, that the occurrence of the punctate opacities were the results of the disturbance caused by an antigen-antibody reaction.

Then, what is their mechanism? Not many references could be found by the Arthus phenomenon (See Table 1), or the neutralization test (See Table 2). They showed only a sensitized condition. Let us follow the courses of the occurrence again. After the last (provocative) injection, first, the conjunctiva and upper lid swelled with congestion. These swellling and hyperemia gradually became more marked up to 3 hours. Then they begin to disappear.

12 to 24 hours later, the secondary diffuse type appears. And within 48 hours, the secondary diffuse type begins to disappear. At times they covered the whole corneal surface. But in the cases of Adenovirus inoculation, it was vestigial. Next, 48 hours later, the secondary marginal punctate type appears.

Finally, 5 to 6 days after the last injection, punctate opacities occur.

These punctate opacities were caused by either the inoculum of Adenovirus type 8,

Table 1. Results of the Arthus Phenomenon with the Rabbits, those were carried out the Successive Subconjunctival Injection

Antigeg	Judging*	No. of rabbits	
		Cases those had punctate opacities	Cases those had no punctate opacities
Inoculum of Adenovirus type 8	+	21, 23, 24, 29,	
	—	16, 32,	18, 20, 22, 25, 26, 30, 31, 33, 36,
Inoculum of Adenovirus type 3	+	80,	72, 78, 79,
	—		71, 73, 74, 75, 76, 77,
Purified Vaccine virus	+		
	—		61, 62, 63, 64, 65, 67, 69, 70,
Bovine serum	+	52, 55, 59, 60,	51, 53, 54, 57, 58,
	—		56,

*Judging; we judged as "+" the cases those had a macula of hemorrhage more than 10×10mm 24 hours after the injection.

Table 2. Results of the Neutralization Test with Sera for the Adenovirus type 8, in the Experiment of Successive Subconjunctival Injection of Rabbits.

	Method of inoculation	Total of inoculum	The number of days from the begining of inoculation	Serum dilution	No. of rabbit's sera	
					Cases those had punctate opacities	Cases those had no punctate opacities
Prior inoculation				<4		16, 18, 21, 22, 23, 24, 29, 30, 32, 33, 41, 43,
After inoculation	Successive subconjunctival injection (0.2 cc once)	1.2 cc	13 days	8	29,	
		1.4 cc	15 days	32	23,	
		1.4 cc	17 days	16— 64	21, 24,	
		1.4 cc	19 days	32—>128	16, 32,	22, 30, 33,
		1.6 cc	28 days	>128		18,
	Once intra corneal injection	0.04cc	20 days	8	37,	
	Successive subconjunctival	1.2 cc	79 days	64	15,	12,
	Intravenous and subcutaneous	43.0 cc	98 days	64		6, 8.

Table 3. Corneal Changes; Those were caused by the Successive Subconjunctival Injection
—Occurrence Rate—

	Groups	Occurrence rate
Primary type	Purified vaccine virus group	80 %
Secondary diffuse type	Bovine serum group	60 %
Secondary marginal punctate type	Bovine serum group	100 %
Secondary punctate type	Adenovirus type 8 group	41 %
	Bovine serum group	40 %

Table 4. Occurrence Rate of the Punctate Opacities. (Secondary Punctate Type)

Inoculum	No. Rabbits tested		Occurrence rate	changes of conjunctiva
	total	cases those had punctate opacities		
Adenovirus type 8	17	7	41%	++
Adenovirus type 3	10	1	10%	+
He-La cell and medium	25	0	0	+
Purified vaccine virus	9	0	0	±±
Bovine serum	10	4	40%	++
Normal saline solution	19	0	0	−

type 3, or bovine serum. Therefore it was thought these punctate opacities were not caused by the affinity of the toxin of the Adenovirus type 8 to the tissue. They were caused by the disturbance of antigen-antibody reaction somewhat like a type of Arthus phenomenon. After the provocative injection, the secondary diffuse type appeared, in the cornea. This also seems to come from a direct disturbance. Then, 5 to 6 days later, the punctate opacities occurred.

If these punctate opacities were also due to direct disturbance, they should appear by 12 to 24 hours after the provocative subconjunctival injection. But at that stage, only the secondary diffuse type was found not the punctate opacities. If the punctate opacities were due to the direct disturbance, it is difficult to understand that a symptom of a direct disturbance by the subconjunctival antigen-antibody reaction, is appearing in the cornea 5 to 6 days after the provocative injection. On the other hand, if the punctate opacities were caused by the antigen-antibody reaction in the corneal tissue, when would the punctate opacities appear? Here the author would like to have you recall the results of the experiment of the second method.

By the intracorneal provocation, the histological observations were as follows. In the corneal epithelium, the changes of the basement cell-nuclei were found to be more severe in the case of one hour after, than in the cases of 12 or 24 hours after the

intracorneal provocation. It seems that the disturbances by the antigen-antibody reaction in the corneal tissue occurred mainly during several hours. These phenomena resemble the cases that were explained by Shikano[22]. (The punctate opacities were not the same as the whole parenchymal opacity in the cornea that was caused by Wessely[23], Szily[24], Arisawa[24], Syoji[25], and Ishida[26]). On the other hand, the punctate opacities could not be caused in 24 hours by the intracorneal provocative injection. The opacities also did not occur during the observations of about 2 weeks, either by means of the single intracorneal injection (non sensitized) of Adenovirus type 8, or by means of the immune-serum injection applying on the same cornea in co-operation with the above injection.

Judging from these results the author arrived at the conclusion that the punctate opacities were not caused by the reaction in the corneal tissue, but caused by the antigen-antibody reaction in the subconjunctival space; Those punctate opacities were the secondary disturbance.

Summary and Conclusion

1. The nonstained nerve fibers are not visible. The nerve branch which is visible without staining is not a nerve fiber, it is a nerve canal.

The nerve canals are visible when the canal current exists, and becomes invisible when the current is stopped. These nerve canals are seen in both the rabbit's eye and in the human eye. In some human cases the nerve canals were seen not only at the large bough but also at the epithelial very fine branches. Thus these evidence show the existence of a circulation system in the corneal parenchyma; which the author wishes to call the nerve canal system.

Through this nerve canal, the liquid current runs at the speed of about 1 mm in 10 minutes towards the corneal center at the principal bough, but slower at the smaller branches. The current becomes more rapid when any stimulus attacks the cornea.

2. There is an unidirectional liquid movement in the corneal parenchyma, and this movement was directed towards the corneal center from the limbus at the speed of under 1 mm in one hour.

3. The punctate corneal opacities of the rabbit's eye are the phenomena of the secondary disturbance caused by antigen-antibody reaction in subconjunctival space using the corneal nerve canals as a medium. "As a medium", means that; there are two ways to reach the punctate opacity from the subconjunctival space through the sclera. One is through the liquid current of the corneal nerve canal, and by this way the liquid runs at the speed of about 1 mm in 10 minutes, having a higher concentration than the following. The other way is through the unidirectional liquid movement in the corneal parenchyma, and by this way the liquid goes at the speed of under 1 mm in one hour, acting surrounding the fine branches of the corneal nerve canal.

If any weak point exists at the fine nerve canal branch; for example, a bifuration,

a point of anastomosis, or a subepithelial tip; the punctate corneal opacity would occur.

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HISTOCHEMICAL FINDINGS ON THE RETINA AFTER SUBCONJUNCTIVAL INJECTION OF ATP-NA

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We investigated histochemically the rabbit's retina after subconjunctival injection of ATP-Na.

Material and Methods

2kg body weight rabbits were used. The rabbit's eye was enucleated at 1,3,6,12 and 24 hours after subconjunctival injection of ATP-Na 5 mg, fixed under 10% formalin solution and stained with Farber's method for succinic dehydrogenase, with PAS test for polysaccharides and with Shimizu-Arizono's method for phosphatase.

Results

Succinic dehydrogenase

At 1 hour after subconjunctival injection, the reaction of the outer part of the retina became weaker. In most of the cases, the reaction of the outer part of all layers of the retina became weaker 3 hours after the injection, and in some cases the reaction of the inner part of the retina became weaker. 6 hours after injection, the outer layers decreasing type was seen much more and the inner layer decreasing type was lesser. After 12 hours have passed, the inner layer decreasing type increased more than at 6 hours. At 24 hours passage, the outer layer decreasing type became as numerous as the inner layer decreasing type.

PAS reaction

At 1 hour after injection, PAS reaction was intense in the internal molecular layer, the external plexiform layer, the intercellular in the external molecular layer, the external limitting membrane and the layer of rods and cones.

At 3 hours past, the inner part of the retina more than the external plexiform layer was filled with glycogen granules.

After 6 hours have passed, the internal limitting membrane and the nerve fiber layer was filled with a clump of glycogen granules and the findings of the internal molecular layer was light and discolored.

After 12 hours of the injection, the clump of glycogen granules became red staining molecular bodies.

24 hours afterwards the PAS reaction in the outer segment became intense.

Table 1. PAS Reaction

	Norm	1hr.	3hrs.	6hrs.		12hrs.		24hrs.
				a	b	a	b	
Int. lim. m.	+	+	+	heavy	fin.	fin.		fin.
Nerv. fib. l.	+	+	+	glyc.	glyc.	glyl.		glyc.
Gang. cell. l.	+	+	+	gr.	gr.	gr.		gr.
Int. ret. l.	++	+	+	+	+	+	+	⋮
Int. mol. l.	↑↓ ++	↓	+	−	−	++	−	⋮
Ext. ret. l.	++	↓	+	±	±	++	+	⋮
Ext. mol. l.	±	↓	↓↓ +	h. g. g.	↑ ++	⋮ ++	⋮ +	⋮
Ext. lim. m.	±	+	+	±	±	⋮ ++	⋮ +	⋮
Inn. segment	±	+	+	±	+	⋮ ++	⋮ +	⋮
Out. segment	+	+	++	++	++	⋮ ++	⋮ +	+++

Table 2. Alkaline Phosphatase

	1hr.	3hrs.	6hrs.	12hrs.	24hrs.
Nerv. fib. l.	±	±~−	+~±	+	+
Gang. cell. l.	++~+	++	++	++	++
Int. ret. l.	±	±~−	+~±	+~±	+~±
Int. mol. l.	++	+++~++	+++~++	+++~++	+++
Ext. ret. l.	+~±	+~±	+~±	+	−
Ext. mol. l.	−	−	−	−	−
Ext. lim. m.	+++	+++	+++	+++	+++
Inn. segment	+~±	±~−	±	±	+
Out. segment	+~±	±~−	±	±	+~±

Table 3. Acid Phosphatase

	1hr.	3hrs.	6hrs.	12hrs.	24hrs.
Nerv. fib. l.	±~−	±~−	±~−	±~−	±~−
Gang. cell. l.	±	+	+	+~±	+
Int. ret. l.	±~−	±~−	±~−	−	++~−
Int. mol. l.	++~+	++	++	++~+	++
Ext. ret. l.	±~−	±~−	±~−	±~−	±~−
Ext. mol. l.	+++~++	+++	+++	+++~++	+++
Ext. lim. l.	−	−	−	−	−
Inn. segment	+~±	+~±	±	++	±
Out. segment	+~±	+~±	+~±	+~±	+~±

Alkaline phosphatase

After 1 hour has passed, alkaline posphatase reaction became intense in the ganglion cell layer, the layer of rods and cones and the external plexiform layer. After 3 hours, the extrenal molecular layer stained blackish-brown. On the contrary, the layer of rods and cones and the internal plexiform layer became weak. After 6 hours, the staining activity in the layer of rods and cones recovered. After 12 hours have passed, the external molecular layer again stained very deep. After 24 hours, all layers of the retina were more light in color than at the passage of 12 hours.

Acid phosphatase

At 1 hour after, all layers of the retina stained deeper than the contrast. After 3 hours, the external molecular layer stained yellowish-brown. The internal molecular and the nerve fiber layer was distinct. After 6 hours had passed, the inner region of

the inner segment stained dark. After 12 hours, the staining activity of all layers of the retina became weaker than at 6 hours. The boundary of each layer of the retina was indistinct. After 24 hours of the injection, every layer of the retina recovered in color.

Discussion

We injected 5 mg of ATP-Na into the rabbits subconjunctiva and observed histochemically its retina. On the succinic dehydrogenase reaction, 1 hour after the subconjunctival injection, there was, for the most part, the outer layer decreasing type. After 3 hours passage, the inner and all other layers decreasing type appeared. As the time after subconjunctival injection went on, the inner layer decreasing type increased. After 24 hours, the inner layer decreasing type increased. After 24 hours, the inner layer decreasing type became almost equal to the outer layer decreasing type in number. On the PAS reaction, after 3 and 6 hours, the inner part of the retina was filled with a clump of glycogen granules. After 24 hours past, the outer segment was stained deep. Namely, in both of the cases of succinic dehydrogenase and PAS reaction, the main part of reaction was not localized in any definit layer, but shifted from the outer part of the retina to the inner part of the retina.

When ATP was added to the extract of the cow retina acceleration of glycogenolysis was appreciated by Suellmann, Vas. Suellmann and Brueckner. Ezuka reported to increase the organic phosphate synthesis of the retina with addition of ATP.

About PAS reaction in this experiment, 3 and 6 hours after injection, the inner part of the retina was filled with a red colored clod and the red staining molecular bodies appeared intercellular on the external molecular layer. Alkaline phosphatase reaction in the layer of rods and cones became weak after 3 hours of the injection. Acceleration of acid phosphatase reaction was checked temporarily after 12 hours. On the whole, however, the staining activity of polysaccharides and phosphatase accelerated gradually.

Summary

Histochemical findings of the rabbit's retina at 1,3,5,12 and 24 hours after subconjunctival injection of ATP-Na 5 mg were the following.

1. As the time after subconjunctival injection went on, the inner layer decreasing type of succinic dehydrogenase reaction gradually increased. After 24 hours, the inner layer decreasing type became almost equal to the outer layer decreasing type in number.

2. One hour after, the intercellular glycogen granules were revealed in the external molecular layer. After 3 hours, glycogen granules occupied rather the inner part of the retina than the external plexiform layer. After 6 hours have passed, the inner part of the retina was filled with a clump of glycogen granules and the findings of the internal molecular layer were light and discolored. Thereafter the clump of glycogen

granules became red, staining molecular bodies.

3. As the time after subconjunctival injection went on, the alkaline phosphatase reaction became intense. The blackish brown staining was obtained in the external molecular layer and the layer or rods and cones after 1 hour, in the nerve fiber layer 12 hours after and in the internal region of the inner segment 24 hours after injection.

4. As the time after subconjunctival injection went on, acid phosphatase reaction became intense. The yellowish-brown staining was obtained in the external molecular layer after 3 hours had past and in the inner segment after 6 hours.

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Fig. 1. Succinic dehydrogenase 6 hours after injection, the outer layer decreasing type.

Fig. 2. PAS reaction 3 hours after injection.

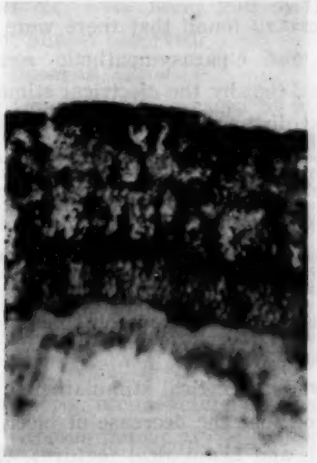


Fig. 3. Alkaline phosphatase 24 hours after injection.

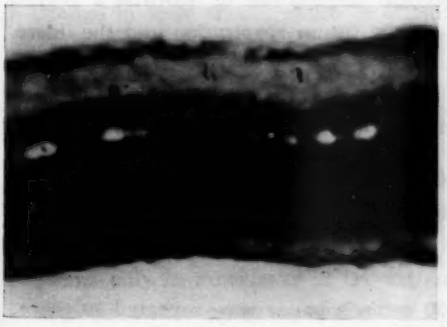
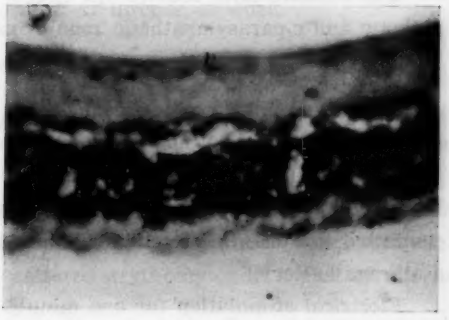


Fig. 4. Acid phosphatase 6 hours after injection.



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THE EFFECT OF ELECTRICAL STIMULATION OF THE HYPOTHALAMUS ON THE TISSUE METABOLISM OF THE RETINA AND ON ITS HYSTOLOGIC FEATURES

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Kurotsu found that there were three kinds of areas: a-parasympathetic, b-sympathetic and c-parasympathetic zones in the hypothalamus. He and his associates reported that by the electrical stimulation of the b-symathetic zone obtatined by placing the coil distance 6-8 cm increased blood pressure, temperature and non protein nitrogen of blood and caused hyperglycaemia, and showed the histological features, that the content of the thyroid gland follicle from there and adrenalin from the adrenal gland were driven out. Furthermore, Kurotsu and his associates observed the rise of intraocular pressure, and the increase of aqueous humor protein etc..

Fukasawa reported recently that by the stimulation of the sympathetic zone decreased the tissue metabolism of ciliary body and its significant hyperamia and edema occured.

By the electrical stimulation to the parasympathetic zone Kurotsu and his associates reported that the decrease of blood pressure and temperature, and hypoglycaemia were caused, and histological features, that the ejection of contents of thyroid gland and of adrenalin of adrenal gland were controlled, were shown. And Fukasawa that the tissue respiration of cilliary body was decreased.

The authors have studied the effects of electrical stimulation of Kurotsu's b-sympathetic and c-parasympathetic zone of rabbits on the tissue respiration and the histologic features of the retina.

Method

The stimulation of the hypothalamus was done by the Kurotsu's method, following Kurotsu's tabulated statistics by attaching electrode-holding apparatus to rabbit's head connected to Porter's induction coils suppled with a current of 2 volts strength from a storage battery.

Electrical stimulation for one minute with 30 seconds pause was repeated 10 times.

The strong stimulation is afforded by placing the coils at the distance of 3-4 cm apart, the weak by 10-12 cm.

The tissue respiration of the retina was measured by the Warburg's indirect method. Histologically hematoxylin-eosin stain was employed.

Results

The change of the general state by the stimulation

By strong stimulation of the b-sympathetic zone, extreme dilatation of pupil, exophthalmos, palpitation, tachycardia etc. appeared and sometimes petechiae in the auricle. These symptoms would be thought as a state of sympathicotonia.

By the weak stimulation above mentioned symptoms were slight, and petechiae in the auricle were not seen.

By the strong stimulation of the c-parasympathetic zone, miosis, moderate dilatation of the auricle vessels, bradycardia, enophthalmos etc. were seen, and by the weak stimulation the change of the general state was not almost observed.

The change of tissue respiration of the retina is shown in TableI-IV

By strong stimulation of the sympathetic zone Qo_2 and $Q_M^{O_2}$ decreased by about 20% immediately and about 15% in one hour after the stimulation, and by weak stimulation the values increased by about 6% immediately and in 30 minutes, and decreased a little in two hours after the stimulation. These changes recovered in about 6 hours or so.

By strong stimulation of the parasympathetic zone Qo_2 and $Q_M^{O_2}$ decreased by approximately 10%, and by weak stimulation the decrease was about 5%. These changes recovered in about 6 hours after the strong stimulation and in 2 hours after weak one.

Histological change of the retina

By the strong stimulation to the sympathetic zone, occured intensive edema at inner layer of the retina and haemorrhage in the vitreous humor at its adjacent part to the ratina (Fig. 1), and by weak stimulation very slight edema on a part of inner layer of the retina, and scattered erythrocytes in a part adjacent to the retina were observed (Fig. 2). When parasympathetic zone was stimulated, retina showed no change in spite of any grade of stimulation, however intense or weak.

Comment

In our experiments as shown in the tables the metabolism of the retina also decreased just like iridociliary body by the strong stimulation of the sympathetic zone, but on the contrary increased by weak.

Histologically the authors observed edema mainly in innerlayer of the retina and haemorrhage of vitreous by strong stimulation of the sympathetic zone, and slight edema only in innerlayer of the retina and scattered erythrocytes in a part of vitreous adjacent to the retina by weak.

Table I. Tissue Respiration of the Retina by the strong Stimulation of the Sympathetic Zone

Time after the stimulation	No. of rabbit	Dried weight of the retina (mg)	Qo_2	$Q_M^{O_2}$
immediately	1	4.366	−11.2	+18.6
		4.186	−14.1	+23.1
	2	5.231	−15.7	+23.0
		4.506	−15.4	+23.6
	3	4.394	−12.6	+24.4
		4.248	−14.0	+24.1
	4	5.306	−15.5	+25.4
		5.585	−11.6	+20.1
	average		−13.8	+22.8
	% of change		decrease 21.1	decrease 21.6
30 minutes	5	5.406	−16.5	+28.6
		5.355	−16.9	+24.9
	6	4.670	−12.4	+25.1
		4.129	−13.7	+26.7
	7	5.559	−14.5	+20.5
		5.153	−14.1	+21.2
	8	5.447	−15.5	+24.9
		5.551	−12.6	+23.3
	average		−14.5	+24.4
	% of change		decrease 17.1	decrease 16.2
1 hour	9	5.369	−16.2	+25.9
		5.396	−16.6	+26.6
	10	5.638	−14.9	+24.0
		5.471	−12.8	+21.0
	11	4.597	−14.3	+22.7
		5.351	−15.0	+24.6
	12	5.264	−14.8	+27.0
		5.406	−14.4	+27.5
	average		−14.9	+24.9
	% of change		decrease 14.9	decrease 14.4
2 hours	13	5.357	−16.7	+25.9
		5.111	−15.1	+29.6
	14	4.259	−18.7	+30.5
		4.441	−17.3	+26.8
	15	5.275	−14.3	+26.4
		4.136	−15.3	+27.1
	16	5.076	−17.8	+26.4
		4.129	−17.1	+27.1
	average		−16.5	+27.5
	% of change		decrease 5.7	decrease 5.5
6 hours	17	4.691	−17.4	+27.8
		4.692	−18.9	+27.7
	18	5.936	−18.5	+33.6
		5.712	−16.5	+30.6
	19	3.625	−17.4	+28.9
		4.673	−19.1	+32.9
	20	3.789	−17.5	+30.1
		4.739	−16.7	+30.3
	average		−17.7	+30.2
	% of change		increase 1.1	increase 3.8
control	average of ten rabbits (No. 21-30)		−17.5	+29.1

Table II. Tissue Respiration of the Retina by the weak Stimulation of the Sympathetic Zone

Time after the stimulation	No. of rabbit	Dried weight of the retina (mg)	Qo_2	$Q_M^{O_2}$
immediately	31	5.660	−18.0	+29.5
		5.321	−17.7	+32.3
	32	4.947	−17.7	+26.7
		4.929	−17.5	+26.8
	33	5.245	−18.0	+28.2
		5.660	−19.5	+32.1
	34	4.912	−18.9	+28.9
		4.703	−20.3	+32.8
	average		−18.5	+29.7
	% of change		increase 5.7	increase 7.2
30 minutes	35	5.351	−19.7	+29.7
		5.534	−19.4	+28.6
	36	5.106	−18.9	+26.9
		5.228	−17.4	+28.0
	37	4.930	−19.6	+30.7
		4.884	−18.4	+28.0
	38	4.697	−20.6	+33.4
		4.831	−19.1	+32.1
	average		−19.1	+29.6
	% of change		increase 9.1	increase 6.9
1 hour	39	4.840	−17.8	+27.6
		4.808	−15.1	+27.0
	40	5.084	−16.3	+27.0
		5.209	−16.4	+24.3
	41	4.328	−18.3	+31.9
		4.285	−18.6	+29.5
	42	5.348	−17.5	+31.3
		5.530	−17.8	+30.5
	average		−17.2	+28.6
	% of change		decrease 1.7	increase 3.2
2 hours	43	4.654	−16.4	+26.3
		4.664	−16.6	+27.0
	44	4.534	−16.8	+30.3
		4.323	−17.2	+28.7
	45	5.764	−17.1	+26.7
		5.664	−17.0	+26.0
	46	5.014	−17.7	+25.9
		5.476	−17.4	+26.1
	average		−17.0	+27.1
			decrease 2.9	decrease 2.2
6 hours	47	4.879	−17.4	+26.7
		4.971	−17.0	+26.5
	48	5.553	−17.8	+30.9
		5.231	−17.1	+31.3
	49	4.794	−17.2	+30.2
		4.755	−17.5	+28.3
	50	4.761	−19.9	+31.3
		5.097	−17.9	+30.6
	average		−17.7	+29.5
	% of change		increase 1.1	increase 6.5
control	average of ten rabbits (No. 51-60)		−17.5	+27.7

Table III. Tissue Respiration of the Retina by strong Stimulation of the Parasympathetic Zone

Time after the stimulation	No. of rabbit	Dried weight of the retina (mg)	Qo_2	$Q_M^{O_2}$
immediately	61	5.168	−15.7	+26.6
		5.239	−13.3	+25.4
	62	4.289	−16.2	+28.2
		3.395	−16.5	+28.3
	63	4.539	−14.2	+26.2
		4.655	−18.0	+31.0
	64	4.347	−17.2	+26.6
		4.398	−13.6	+24.1
	average		−15.6	+27.1
	% of change		decrease 11.9	decrease 4.6
30 minutes	65	5.692	−16.7	+29.0
		4.738	−16.9	+27.8
	66	5.507	−15.9	+25.9
		5.623	−14.2	+22.6
	67	4.488	−16.3	+26.3
		4.546	−16.0	+24.8
	68	5.366	−16.5	+25.0
		5.572	−16.2	+24.7
	aberage		−16.1	+25.8
	% of change		decrease 9.0	decrease 9.2
1 hour	69	5.053	−11.4	+22.8
		5.059	−15.9	+26.1
	70	5.331	−16.6	+30.5
		5.627	−16.2	+28.8
	71	5.318	−16.8	+24.7
		5.264	−16.6	+24.7
	72	5.674	−16.8	+26.7
		5.639	−16.5	+25.9
	aberage		−15.9	+26.3
	% of change		decrease 10.2	decrease 7.4
2 hours	73	5.022	−16.2	+26.4
		4.901	−16.6	+27.4
	74	5.095	−15.4	+27.2
		4.624	−15.0	+21.7
	75	3.309	−18.8	+26.7
		3.777	−16.6	+27.5
	76	5.207	−18.8	+30.3
		4.992	−17.1	+29.9
	average		−16.8	+27.2
	% of change		decrease 5.1	decrease 4.2
6 hours	77	5.495	−18.3	+27.9
		4.548	−15.7	+26.2
	78	4.756	−15.3	+27.0
		4.125	−18.5	+27.9
	79	3.641	−17.2	+29.8
		3.534	−14.5	+25.4
	80	5.066	−17.2	+30.8
		4.859	−16.9	+29.7
	average		−16.7	+28.1
	% of change		decrease 5.6	decrease 1.1
control	average of ten rabbits (No. 81-90)		−17.7	+28.4

Table IV. Tissue Respiration of the Retina by weak Stimulation of the Parasympathetic Zone

Time after the stimulation	No. of rabbit	Dried weight of the retina (mg)	Qo_2	$Q_M^{O_2}$
immediately	91	4.426	−16.0	+25.6
		4.487	−15.8	+26.2
	92	4.510	−14.8	+26.2
		4.796	−14.4	+26.3
	93	5.445	−16.9	+26.5
		5.042	−16.8	+25.6
	94	5.359	−17.2	+27.5
		5.202	−16.4	+28.3
	average		−16.0	+26.5
	% of change		decrease 6.4	decrease 5.7
30 minutes	95	5.329	−16.5	+24.8
		5.374	−16.4	+27.9
	96	5.399	−16.3	+24.8
		5.388	−17.2	+27.3
	97	4.585	−16.2	+26.5
		4.948	−16.5	+25.6
	98	4.614	−17.5	+27.6
		4.412	−15.8	+26.9
	average		−16.4	+26.4
	% of change		decrease 4.1	decrease 6.0
1 hour	99	4.934	−16.0	+25.0
		4.922	−15.5	+24.3
	100	5.137	−17.0	+25.4
		5.285	−16.1	+25.4
	101	5.163	−16.4	+25.8
		4.427	−16.8	+27.2
	102	4.897	−15.8	+25.4
		4.958	−16.2	+28.3
	average		−16.2	+25.9
	% of change		decrease 5.3	decrease 7.8
2 hours	103	5.047	−18.0	+27.8
		4.946	−17.8	+25.7
	104	4.717	−17.6	+28.4
		4.304	−17.6	+28.5
	105	4.225	−17.0	+26.5
		4.152	−16.5	+26.0
	106	4.777	−16.3	+31.2
		4.786	−16.7	+30.2
	average		−17.2	+28.0
	% of change		increase 0.6	decrease 0.4
6 hours	107	5.314	−16.8	+29.6
		5.537	−16.6	+25.2
	108	4.641	−16.8	+29.7
		4.674	−17.2	+29.2
	109	4.562	−16.3	+32.4
		4.594	−17.5	+26.9
	110	3.567	−17.1	+31.0
		3.821	−17.2	+30.6
	average		−16.9	+29.3
	% of change		decrease 1.2	increase 4.3
control	average of ten rabbits (No. 111-120)		−17.1	+28.1

Fig. 1. Histologic changes of the retina and the vitreous by the strong stimulation of sympathetic zone. ×300.

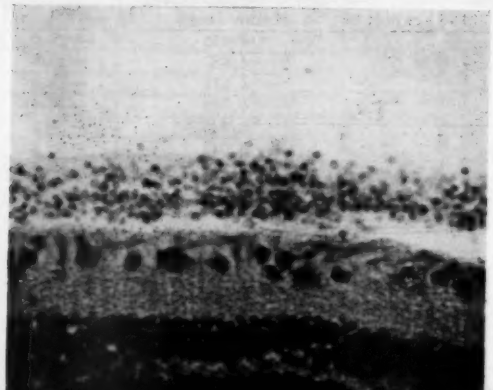
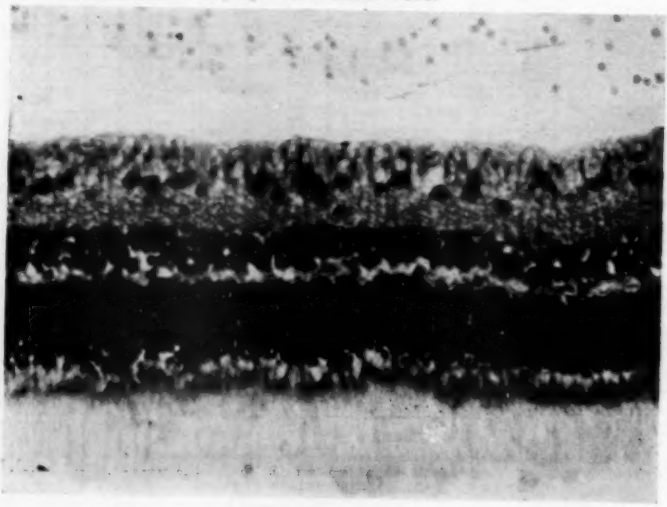


Fig. 2. Histologic changes of the retina and the vitreous by the weak stimulation of sympathetic zone. ×300.



It is easily assumed that these histologic changes occured by strong stimulation of sympathetic zone will cause the decrease of metabolism of the retina. But by the weak stimulation the tissue respiration increases in spite of the histological change in the inner layer of the retina. Because Kurachi, Saito and Kozawa had already reported that the tissue metabolism of the inner layer of the retina was not so great as its outer layer. Further, according to reports above mentioned, general metabolism must be increased by hyperfunktion of thyroid gland and therefore the metabolism of the retina will be increased as a whole, though a slight decrease of tissue respiration of the inner layer may occur.

It is supposed that these histologic changes were caused by the rise of blood pressure, as Takagi and Takemura, and also Fukasawa maintained. These damages in the retina exist mainly in the inner layer by strong stimulation and only in the inner layer by weak. It is assumed that the change is due to the fact that the inner layer of the retina is nourished only by the arteria centralis retinae which shapes the arteria terminalis, and so is easily influenced by the rise of blood pressure, and that on the other layer controlled by the choroid which has an abundance of vessels and lymphatic ducts, and consequently is not so directly influenced by the sudden or intense change of blood pressure. Furthermore, the histological change indicates that haemorrhage and other lesions of the retina caused by hypertension occur more easier in the inner layer than in the outer. By our experiments the tissue respiration of the retina was decreased also by strong or weak stimulation of the parasympathetic zone.

Summary

Stimulating electrically the Kurotsu's b-sympathetic and c-parasympathetic zone of rabbits, the authors obtained the following results.

1. In case of the stimulation of the sympathetic zone, the tissue metabolism of of the retina showed about 20% decrease immediately after the strong stimulation and about 10% increase immediately after the weak. Histologically intense edema mainly in the inner layer of the retina and haemorrhage in the vitreous humor at the adjacent part to the retina were observed by the strong stimulation and very slight edema only in the inner layer of the retina and scattered erythrocytes in the vitreous at the adjacent part to the retina were observed by the weak.

2. In case of the stimulation of the parasympathetic zone, the tissue metabolism showed about 10% decrease after the strong stimulation and about 6% decrease after the weak. Histologically remarkable changes were not observed.

(Published in Acta O. Jap. 61: 2490, 61:2499, 1957 and 63:823, 1959 in Japanese.)

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EXPERIMENTAL ROENTGEN-RAY CATARACT

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Experiment I: Many slight radiation cataracts were discovered among the survivors of the atomic bomb explosion in Nagasaki. Therefore, a project to cause the same slight roentgen-ray cataract in rabbit and examine the cataract both clinically and

Table 1. Clinical Findings in Rabbit

Radiation Dosage	Animal Number	Period Observed (days)	Anterior Subcapsular Vacuoles		Diffuse Opacities in the Anterior Cortex		Vacuoles at the Equator	
			Latent Period (days)	Maximal Severity	Latent Period (days)	Maximal Severity	Latent Period (days)	Maximal Severity
45 r	1	427		−		−		−
	2	427		−		−		−
90 r	3	315		−		−		−
	4	725		−		−		−
200 r	5	392		−		−		−
	6	427		−		−		−
350 r	7	245		−		−		−
	8	455		−		−		−
500 r	9	392		−		−		−
600 r	11	364	63	+		−		−
	12	847		−		−		−
800 r	15	728	98	+		−		−
900 r	16	364	98	+		−		−
	17	1092	126	++		−		−
1000 r	18	427	112	++		−		−
	19	427	70	++		−		−
1300 r	20	364	98	++		−		−
	21	245	56	++		−	84	+
1500 r	23	329	91	+++		−	49	+
	24	301	77	+++		−	35	+
2000 r	29	364	70	+++	105	+	56	+++
	30	210*	42	+++	126	+++	35	+++

* Cataract became complete.

histologically was conceived. Rabbits, three to five months old, were irradiated with 45r to 2000r of X-ray (160 kvp.; 3 ma.; 0.5 mm. Cu+1.0 mm.Al). Clinical observations were made on the lenses seven months to there years, after which the lenses were removed, fixed with 10% formalin, embedded in celloidin and stained with hematoxylin-eosin and van Gieson's stain, for hislological examination.

Experiment II: This experiment was undertaken to determine whether the difference between the reported results of experimental roentgen-ray cataracts by other

Lenses after X-irradiation.

Posterior Subcapsular Vacuoles		Opacities of the Posterior Suture		White, Punctate Opacities in the Posterior Cortex		Posterior Subcapsular Plaque-like Opacities		Tuff-like Opacities in the Posterior Cortex	
Latent Period (days)	Maximal Severity	Latent Period (days)	Maximal Severity	Latent Period (days)	Maximal Severity	Latent Period (days)	Maximal Severity	Latent Period (days)	Maximal Severity
154	+		−		−		−		−
140	+		−		−		−		−
154	+		−		−		−		−
133	+		−		−		−		−
126	+		−		−		−		−
119	+	154	+	154	+		−		−
119	+	147	+	147	+		−		−
147	+	168	+	168	+		−		−
119	++	133	++	133	++	133	+		−
112	++	147	++	147	++	147	+		−
133	++	168	++	168	++	168	+		−
98	++	126	++	126	++	126	++		−
105	++	126	++	126	++	126	++		−
133	+++	161	+++	161	+++	161	++	224	+
112	+++	133	+++	133	+++	133	+++	203	+
105	+++	126	+++	126	+++	126	+++	182	+
112	+++	126	+++	126	+++	126	+++	154	++
91	+++	105	+++	105	+++	105	+++	126	++
105	+++	133	+++	133	+++	133	+++	161	++
91	+++	105	+++	105	+++	105	+++	147	++
70	+++	105	+	105	+	105	+++	140	+++
70	+++	98	++	98	++	88	+++	140	+++

authors and those of experiment I was due to the difference in the hardness of X-ray. Young rabbits were used. The first group was irradiated with 600r to 2000r of X-ray (80 kvp.; 3 ma.; 2 mm.Al), and clinically observed during eleven to twenty-three months. After the observation, the eyeballs were removed and the lenses were histologically examined, using the same method as described in Experiment I. The second group was irradiated with 800r of X-ray (180 kvp.; 20 ma.; 0.2 mm.Cu+0.5 mm.Al), and clinically observed for one year. After these examinations, the results obtained from the two groups were compared with that of Experiment I.

Table 2. Histological Findings of

Radiation Dosage	Animal Number	Days after Irradiation	Migrated Cells beneath the Posterior Capsule	Undifferentiated Lens Fibers in the Posterior Cortex	Balloon-cells in the posterior Cortex
45 r	1	427	+	+	−
	2	427	+	+	−
90 r	3	315	+	+	−
	4	728	+	−	−
200 r	5	392	+	+	−
	6	427	+	+	+
350 r	7	245	+	+	+
	8	455	+	+	+
500 r	9	392	++	+	++
600 r	11	364	++	+	++
	12	847	+	+	+
800 r	15	728	++	+	++
900 r	16	364	++	+	++
	17	1092	+	+	+
1000 r	18	427	++	++	++
	19	427	++	++	++
1300 r	20	364	+++	++	+++
	21	245	+++	++	+++
1500 r	23	329	+++	++	+++
	24	301	+++	++	+++
2000 r	29	364	++++	+++	++++
	30	210	++++	+++	++++

In order to test the validity of the biomicroscopic observation, some of the fresh lenses, before they were fixed with 10% formalin, were observed with the stereomicroscope.

Experiment III: Physiological opacities of the normal lenses of untreated rabbits were clinically and histologically examined to compare with slight lenticular changes of the rabbits irradiated with lower doses of X-ray. One hundred and eighty-three rabbits (three months to one year old) and 10 rabbits (two to three years old) were used.

Roentgen-Ray Cataracts in Rabbits

Displacement of the Nuclei in the Lens Bow	Swelling of the Lens Fibers in the Posterior Cortex	Degeneration of the Epithelial Cells	Collapse of the Lens Fibers in the Posterior Cortex	Swelling of the Lens Fibers in the Anterior Cortex
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
−	−	−	−	−
+	±	−	−	−
+	±	±	−	−
+	±	±	−	−
+	+	+	−	−
+	+	+	−	−
++	+	+	+	−
++	++	++	+	−
++	++	++	+	−
++	+++	++	+	+
++	+++	++	+	+
++	+++	++	+	+
++	+++	++	+	+
++++	+++	++++	++	++
++++	++++	++++	+++	+++

Results

Experiment I: The results are shown in the following tables.

Experiment II: Clinically, in the first group, anterior subcapsular vacuoles, white punctate opacities in the anterior cortex, posterior subcapsular vacuoles, plaque-like opacities in the posterior subcapsular region, opacities of the posterior suture itself and white, punctate opacities in the posterior cortex from 600r, and in addition to these changes tuff-like opacities in the posterior cortex from 900r were seen. The lens became totally opaque with irradiation of 2000r.

Histologically, degeneration of the epithelial cells, displacement of the nuclei in the lens bow, migrated cells beneath the posterior capsule, balloon-cells in the posterior cortex and swelling of the lens fibers of posterior cortex were seen from 600r. These clinical and histological findings were similar to those found in Experiment I in regards to the latent period and to the severity.

Clinical findings of the second group were almost similar to those of the rabbits irradiated with 800r in Experiment I and in the first group of Experiment II in which tuff-like opacities in the posterior polar cortex were detectable over 900r, but not under 800r. Tuff-like opacities were not seen in the second group. Comparison of the findings

Table 3. Relationship Between Quality of X-radiation and Latent Period of Cataract

Half Value Layer (mm. Cu)	X-ray Factors	Experiment Number	Animal Number	Latent Period of Plaque-like Opacities
0.091	80 kvp.; 3 ma. 2 mm. Al	II First Group	33	21 weeks
			34	21 weeks
0.823	160 kvp.; 3 ma. 0.5 mm. Cu+1.0 mm. Al	I	14	19 weeks
			15	18 weeks
0.852	180 kvp.; 20 ma. 0.2 mm. Cu+0.5 mm. Al	II Second Group	40	18 weeks
			41	20 weeks

of these three groups is shown in the following table.

From the results of this experiment, it appeared that hardness of X-ray might not have any effect on the occurrence of roentgen-ray cataract.

Experiment III: Physiological opacities of the lenses of the rabbits were seen in 180 out of 366 eyes (49.1%).

Classification and frequency of opacities:

Persistent pupillary membrane	3.0%
Punctate opacities in the anterior cortex	1.6%
Opacities of the anterior suture	0.3%
Nuclear cataract	5.9%
Punctate opacities in the posterior suture within the nucleus	1.6%
Punctate opacities in the posterior cortex	16.0%
Punctate opacities in the posteroior suture within the cortex	13.3%

Posterior subcapsular vacuoles	2.5%
Linear opacities in the equator	0.8%
Opacities on the surface of the posterior capsule	6.8%

A large opacities on the surface of the posterior capsule was examined histologically and determined to be persistence of the tunica vasculosa lentis.

In the examination of the posterior subcapsular vacuoles of ten adult rabbits (2-3 years old), the vacuoles were seen in eight eyes of six rabbits. It was difficult to differentiate these vacuoles morphologically from the vacuoles caused by lower dose of X-ray. The number of vacuoles in the control lenses ranged from one to eight, and they were scattered without any relation to the posterior suture. On the other hand, the number of vacuoles in the lenses of rabbits irradiated witn 45r of X-ray was over 20, and they were scattered along the posterior suture. From these two specific findings, the vacuoles caused by X-ray were differentiated from those of the control.

Five lenses of five rabbits that had a few vacuoles in the posterior subcapsular region were histologically examined. In these, a migrated cell beneath the capsule of the posterior pole was seen in one lens. Since the cell was swollen, it was regarded as a degenerated cell. None of the lenses showed subcapsularly migrating cell from the equator to the posterior pole, balloon-cell, or displacement of the nuclei in the lens bow. A few young fiberswere seen in the posterior cortex in the lens mentioned above and in two others.

Conclusion

1. An irradiation dose as low as 45r caused roentgen-ray cataract in the rabbit lens, both clinically and histologically. The minimum dose of cataractogenic X-ray was much lower, compared with those reported in the literature. This fact may be due not to the hardness of X-ray, the age of animal, and the costitution of the individual, but to the improvement in the examination-technique.

2. The histological changes caused by 45r or 90 r of X-ray were abnormal migration of epithelial cells and undifferentiated fibers into the posterior cortex. With the exposure of 200r or 350r, a few, small balloon-cells were also observed in the posterior cortex. In all the eyes exposed to 500r or more, a subcapsular accumulation of debris at the posterior pole was clearly seen. This histological abnormality seemed to be identical with the subcapsular plaque-like opacities at the posterior pole which had been seen with the slit lamp.

3. The severity of the roentgen-ray cataract was proportional to the radiation dosage, and the latent period of the cataract was inversely proportional to the dosage. Progression of opacities was restricted within the dosages. These facts were also seen in the atomic-bomb radiation cataracts in Nagasaki.

4. Rabbits irradiated with 600r, 800r, and 900r were observed for 28, 24, and 36 months, respectively. Both clinically and histolgically, cataractous changes seemed to

regress slightly toward the end of the observation period. However, it can not be concluded that the atomic-bomb radiation cataract will take the same course as the experimental cataract, because in the past ten years since the explosion not a case in which the opacities regressed was seen.

5. In rabbits which were exposed to 900 r and which had tuff-like opacities in the posterior polar cortex, epithelial cells of the germinative zone were still unable to differentiate into normal fibers even after the lapse of three years since irradiation.

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CLINICAL AND EXPERIMENTAL STUDIES ON OCULAR MANIFESTATIONS OF THE DIFFUSE COLLAGEN DISEASES

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Prephase

A certain series of the lesions such as periarteritis nodosa, disseminated lupus erythematosus, diffuse scleroderma, dermatomyositis, rheumatic fever, rheumatoid arthritis, diffuse glomerulonephritis and malignant nephrosclerosis, etc., in generally accumulated under the category of so-called collagen diseases, is accepted as an acute or chronic systemic disease, designated pathohistological changes, characterized by peculiar fibrinoid swelling and/or degeneration and even necrosis of the angio-connective tissues.

These series of the diseases, as Klemperer and others who first initiated this concept, represent a common histological alteration of the angio-connective tissues, characterized by fibrinoid degeneration, however their clinical manifestations are suitably often variable or are some times quite different case by case. This is due likely to either some factors in accordance with the kinds of organs invaded, or that of one of which, vascular system or connective tissue would mainly be involved, as well as that of its mode of progress.[15]

The concept of the collagen diseases has principally been conducted by pathologists only from the pathohistologic view points. Therefore, it is suitable that clinical considerations would be equivocal and occasionally not coincident. Later, it is generally said that peculiar type of vasculitis is identified as seen in these series of the diseases,[10][17][18] and the vascular lesions are closely reflected in clinical manifestations.[9]

The author investigated these series of the diseases on the selected cases as well as autopsy cases among others.

This series of the diseases may, as is well known, often associate with ocular findings which may sometimes be a preexisting or sometimes be a initial sign of the diseases. Additionally, as ocular lesion would parallelly progress with the generalized

process of the disease or sometimes would prevail over, it may be valuable for considering prognosis of the disease. This fact indicates that ocular signs in these diseases are most important for ophthalmologists.[8)14)]

First, the author presented both clinical and pathological findings on 4 autopsy cases, regarded as a series of the collagen diseases, and discussed especially on ocular manifestations.

Second, experimental investigation was done to analyse on the ocular change and its relationship to the generalized lesion in cases of experimentally produced periarteritis-nodosa-like vascular lesion.

Observations

A) Clinico-pathological studies were made on 4 cases. The results were as follows:

1) The 1st case was a 24 year old male. Clinical diagnosis was chronic glomerulonephritis. He expired of uremia. Rheumatoid vasculitis was disclosed by postmortem examination.

The 2nd case was a 17 year old female. Clinical diagnosis was chronic glomerulonephritis. She died of also uremia. Postmortem pathological examination revealed malignant nephrosclerosis which complicated with chronic glomerulonephritis.

In both cases, main ocular lesion was identified as a retinitis albuminurica clinically as well as from the pathological aspect. (Figs. 1 and 2)

Renal lesion of both cases was diffuse glomerulonephritis and partly suggested a malignant nephrosclerosis. Additionally, in the 1st case, rheumatoid or periarteritis-nodosa-like vascular lesion was present in various organs and tissues, such as the

Fig. 1. (1st case) The retina showing edema, hemorrhage in each layer and into the subretinal space. Proliferation of glia tissue and detachment seen. (H. & E. Stain)

Fig. 2. (2nd case) The retina and choroid showing edema, hemorrhage in the nerve fibre layer, and fibrinoid swelling of the choroidal arteriole associated with swelling of the pigmentepithelium layer and perivascular fibroblast infiltration. (H.& E. Stain)

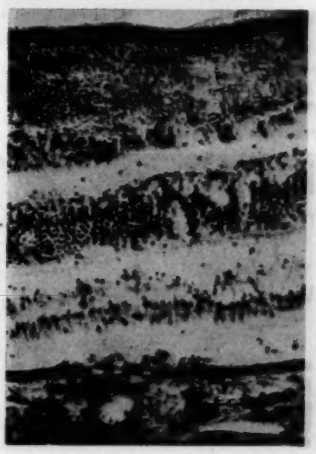


Fig. 3. (1st case) The kidney showing vasculitis obliterans of the lobular artery. (E.v.G. Stain)

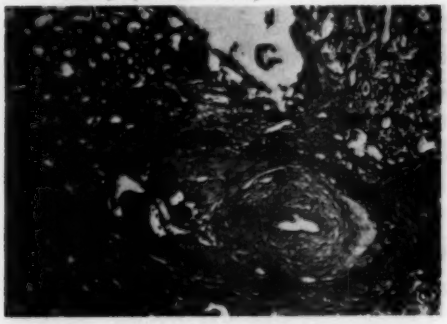


Fig. 4. (2nd case) The liver showing fibrinoid degeneration of a artery in the Glisson's sheath. (H. & E. Stain)

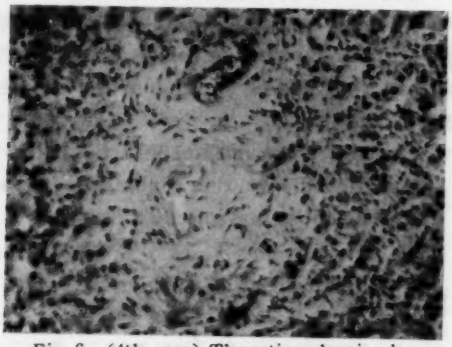


Fig. 5. (3rd case) The retina showing edema, perivascular cell-infiltration (right) and hemorrhage (left). (H. & E. Stain)

Fig. 6. (4th case) The retina showing large aggregation of cytoid bodies of the nerve fibre layer. (H. & E. Stain)

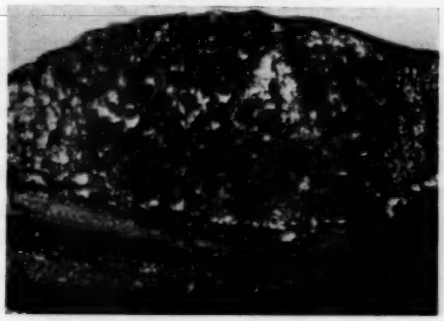


Fig. 7. (3rd case) The left axillary lymph node showing fibrinous swelling of a vessel wall. (H. & E. Stain).

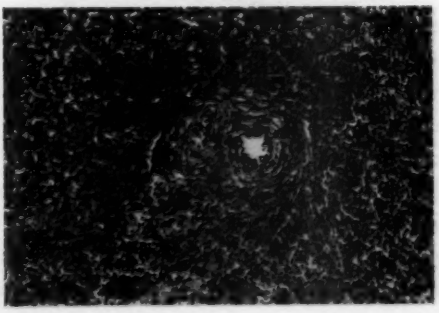
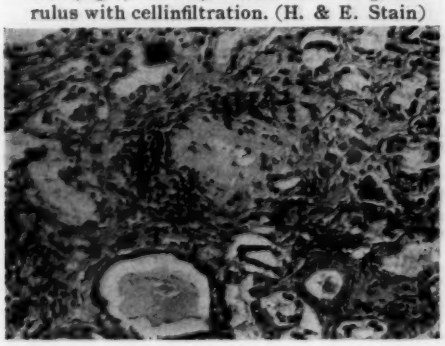


Fig. 8. (4th case) The kidney showing hyaline-fibrinoid degeneration of a vasa afferens (right) and hyalinization of a glomerulus with cellinfiltration. (H. & E. Stain)



kidney, adrenal, pancreas, brain, testis and knee joint, etc., as well as subconjunctival tissue, episclera and sclera. (Fig. 3) In the 2nd case, fibrinoid degeneration of the vessel wall was demonstrated, widely spread in various organs, such as the kidney, liver, pancreas, jejunum and adrenal, as well as choroid of the eye. (Figs. 2 and 4)

2) The 3rd case was a 30 year old female. Clinical diagnosis was disseminated lupus erythematosus complicated with alopecia areata and hypochromic anemia. An actual cause of death was massive and multifocal haemorrhage of the lungs. Pathological diagnosis was concluded unusual type of disseminated lupus erythematosus.

The 4th case was a 40 year old female. Clinical diagnosis was pemphigus acutus adultorum, acute nephritis and sepsis. She died of bronchopneumonia. Postmorten examination revealed pemphigus acutus adultorum complicated with some lesions suggestive of allergic etiology in various organs.

In both cases, main ocular lesion was consistent with toxic retinopathy, ophthalmoscopically and histopathologically. (Figs. 5 and 6)

In the 3rd case, fibrinous swelling of the vessel wall was demonstrable in the heart, ileum, lymph nodes and urinary bladder, etc. (Fig. 7) In the retina, perivascular cell infiltration, consisted of lymphcytes, plasmacells and polymorphonuclear leucocytes was detected. (Fig. 5) In the 4th case, hyaline-fibrinoid degeneration of the vascular system was seen in the kidney, lung and skin, etc. (Fig. 8) In the retina, edema of the perivascular space was seen.

B) Experimental study was done expecting to produce a periarteritis-nodosa-like vascular lesion. Twenty Wistar albino rats were operated upon by a simple device of unilateral nephrectomy and another side of the kidney wrapped with silk(Page's method).[5)12)13)] Sequental observation covered a period from 4 to 7 months.

The results were as follows.

1) Hypertrophy of the heart was observed in 19 rats, 7 of which showed vascular lesion. The capillaries in the periadrenal tissue, pancreas, peripancreatic tissue and

Fig. 9. (rat) The pancreas showing intimal hypertrophy of capillaries with perivascular cellinfiltration (H. & E. Stain)

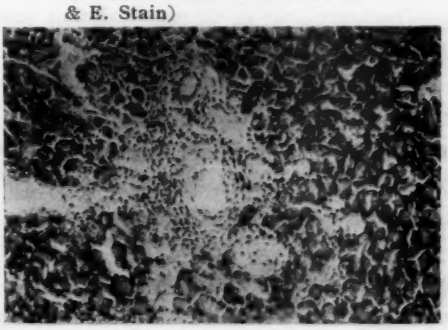


Fig. 10. (rat) The peribronchial tissue showing degeneration of the wall of a small artery (right lower). (H. & E. Stain)

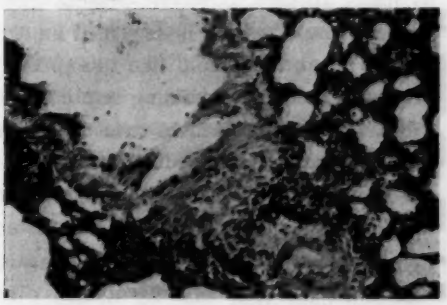


Fig. 11. (rat with angitis) The right eye showing a extensive hemorrhage of the retina, partly extending to the internal reticular layer and into the vitreous. (H. & E. Stain)

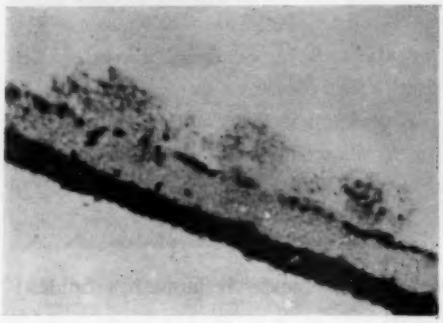
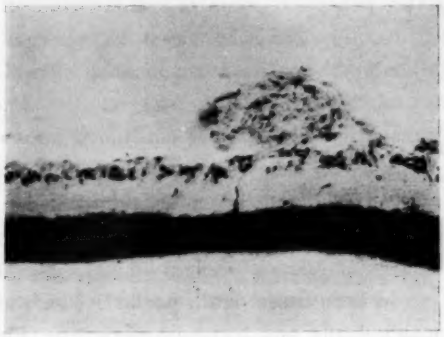


Fig. 12. (rat without angitis) The right eye showing a mass of organizing blood clot in the vitreous. (H. & E. Stain)



mesentery, bronchial arterial branches or pulmonary arterioles were involved, showing intimal hypertrophy, intimal swelling or degeneration of the wall with perivascular cellinfiltration of lymphocytes, plasmacells or monocytes. (Figs. 9 and 10)

2) Among 7 rats, representing vascular lesion, 5 animals had mainly diapedetic or partly ruptured hemorrhage in the retina, choroid, iris or into the vitreous. (Fig. 11) No definite vascular lesion was detected. Additionally, 3 rats among 12, showing hypertrophy of the heart with vascular lesion, had similar hemorrhage in the retina as well as that into the vitreous or subretinal space. (Fig. 12)

Comment

Clinicopathological observations are summarized as follows:

Main ocular lesion of the lst and 2nd case is a retinitis albuminurica, and the vascular lesion of both cases appears to have a close relationship to that of the glomerular tufts and of the arterioles of the kidney, further more that seen in various other organs.

Many opinions of the pathogenesis of the retinitis albuminurica have been reported by many authors, however, present time two theories appear to be mainly accepted, in general, one of which is regarded as a toxic etiology and another one is hemodynamic one. The author would like to support the latter theory from our observations, but problems are still remaining and additional study is necessary in this concern.

It is no doubt that in case of retinitis albuminurica glomerulonephritis or malignant nephrosclerosis would be considered as a so-called diffuse collagen disease, even if the lesion be limited to the kidney or it associate with periarteritis nodosa, disseminated lupus erythematosus, rheumatismus.[15]

This concept may lead us that exact and systematic examinatons of the patient and follow-up for detecting the collagen diseases are important, when we observe such a

patient with retinitis albuminurica.[16]

In the 3rd and 4th case, main ocular lesion is identified as a toxic retinopathy. Toxic retinopathy of the 3rd case preceded to acute aggravation of the generalized condition and it progressed along the patient's condition and deteriolated. In the 4th case, ocular findings appeared simultaneously with generalized disease.

Ophthalmoscopic findings, as well as improvement of such findings, especially increasing or decreasing of cotton wool patches, are clinically important to estimate the following generalized pathological changes or presage as an indicator.[3][7]

On the pathogenesis of toxic retinopathy, there are two different theories, vascular and toxic, reported.[1][7] In both cases, vascular lesion of the retina appears to be quite similar to that generally seen in various organs. Therefore, this finding should be interpreted as a partial phenomenon of generalized vascular lesion, if it be direct sequels or indirect.

On the pathogenesis of cytoid body mainly seen in toxic retinopathy, several theories are published.[8] Transudation of serous fluid from blood stream caused by vascular change occurred in the retina appears to produce an edema of the supporting tissue of the nerve fibre layer in keeping with its original structures and follow swelling of neuroglia fibres. These changes are varied in degree from slight edema to some extent forming a mass of cytoid bodies in the nerve fibre layer and their transitional findings even microscopic bleeding are often detectable.

Experimental datas are summarized as follows:

The nature of vascular lesion of the rats which had been unilaterally nephrectomized and another one wrapped with silk was resembled to human necrotizing angitis, reported by Zeek.[17] The pathogenesis of these vascular lesions appears to have some relationship to hypertension, because all of the animals with angitis showed more or less hypertrophy of the heart in the present experiment.[2][5]

Ocular lesions seen in the rats with vascular lesion were hemorrhages of the retina, choroid, iris and that into the vitreous. These findings may resemble to that seen in the human fundus hypertonicus.[4] Although no definite vascular lesion was detected in the retina, choroid and iris, etc., loosening of some perivascular spaces was seen and it may resemble to the hypertensive arteriolopathy (Scheinker).[11]

Ocular lesions seen in the animals without angitis were also hemorrhages of the retina and that into the vitreous or subretinal spaces.

These hemorrhages in both groups, with and without angitis, are quite similar and these ocular findings should be regarded as a fundus hypertonicus, because all of the animals showed hypertrophy of the heart.

Relationship between ocular lesion and degree of generalized lesion will be discussed here.

Hypertrophy of the heart in the animals with vascular lesion was not so high grade, as comparing with that without angiitis. It is seemed to be fact that high degree

of hypertension was not always a prerequisite factor to produce a vascular lesion, but some investigators mentioned that rapid elevation of blood pressure might be necessary.[2)]

Close relationship was absent between occurrence of ocular lesion and degree of hypertrophy of the heart. However, it was fact that renal lesion in the group witl. ocular lesion was more severe than that failed or lack in ocular lesion. It is generally said that considerable relationship is present between occurrence of vascular necrosis in the brain and the degree of glomerular lesion in the kidney according to the rabbits operated by Goldblatt's procedure.[6)] This fact may lead a quite correlation in pathology of ocular and cerebral vessel.

The present experiment was successful to produce a periarteritis-nodosa-like vascular lesions in various organs, however ocular lesion was not conincident to these changes, but it was a lesion which was secondary to hypertension considered as a fundamental condition producing vascular lesion.

Summary

Ocular manifestations of the cases regarding as a so-called collagen disease and of the rats experimentally produced necrotizing angitis, were presented here.

Main ocular lesion was a retinitis albuminurica, toxic retinopathy and fundus hypertonicus. The pathogenesis, diagnostic value and prognostic significance of the lesion were discussed.

These seem to be most valuable for observation of ocular lesions of various diseases from a different view point of a concept of the collagen diseases. The author emphasized concerning a correlation between ocular change and the collagen diseases characterized by generalized vascular lesion.

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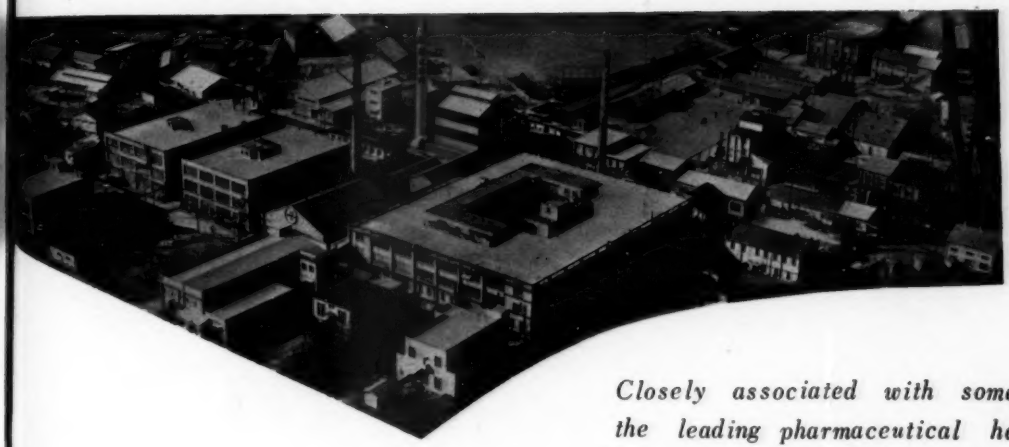

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